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CHILD HYGIENE AMONG THE AMERICAN INDIANS:

A Chapter in Early American Pediatrics

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When one of the primitive inhabitants of this continent took sick or was ailing or injured, there were many and diverse resources to which he could turn for aid and comfort. The old women of the tribe, with their traditional lore handed down by word of mouth through the years of trials and tribulations, were called upon for aid and advice. Then there were medicine men and shamans available when more erudite help was required or desired. According to Sahagun, the tlamatquiticitl gave advice and care in labor and in the management of infants among the ancient Aztecs of Mexico. As usual with all primeval races, religion and superstition played a large part in the healing ritual, yet much was based upon the empiric knowledge gained by generations of trial and error, the memory of which became engrafted on the phylogenetic mind. The skill of the American Indians in preparing and administering herbs is traditional. Right here in my native city Indian herb doctors still ply their trade, strings of dusty, drying plants prominently displayed in windows and hanging from various points of view. When a child was sick the Indians invoked the same measures as a rule as were generally used for the grownup: herbs, "simples," as they were apt to be called by the early colonists, sweat baths, depletives in the form of emetics, purges, venesection, scarification, sucking, a very common procedure widely used among the American aboriginals of both hemispheres and curiously akin to cupping so popular the world over, and to leeching, too, since the sucking often was continued until blood was drawn. Sacrifices to the spirits of evil for appeasement, or to the benevolent spirits in supplication, and offerings to the various deities differed only in detail but

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not in primitive ideology from practices of like import among the Greeks, let us say, with their votive offerings or the African savages with their gifts to the spirits that enliven the environment about them.

If there was no specialization of pediatric practice among the Indians, nevertheless there were many facts to be gleaned from the literature which should be of particular interest to those concerned with child welfare.

Concerning the primordial state of the inhabitants of the Western hemisphere it is of course difficult to determine much more than is conjectural. Most of our knowledge about these prehistoric peoples derives from a study of skeletal remains, and students of paleopathology are unhappily lacking in unanimity concerning the interpretation of osseous changes found in pre-Columbian remains. However, such study is not entirely devoid of value, even if at times productive of conflicting opinion. In examining hundreds of skulls of ancient pre-Columbian Peruvians at the National Museum at Lima, Dr. William T. Corlett of Cleveland, Ohio, was amazed at the unusual amount of skull deformation brought about by binding the head in infancy, while the bones were still soft and pliable, until the desired shape was produced (Corlett, 1935). In a cemetery of four hundred mummies spoken of as the "great Necropolis" in the slope of the Cerro Colorado all the skulls were found to have been artificially elongated. This custom of shaping the heads of Indians is, and probably was, done for cosmetic purposes and was widely practiced throughout the world.[1] The belief of the American Indian in the ability to mold and shape the growing body to the desired form is curiously illustrated in the tale of Francisco Chicorana. He

[1]I can still remember my mother advising her friends with young infants to mold their skulls gently so that they should have "nice, round heads." The so-called Thessalic method of binding was condemned by Soranus as cruel and burdensome. This method, similar to that of the American Indians, must have been a common primitive one. Thracians and Macedonians also bound the infants to a board.

Hippocrates in Chap. XXI of Airs, Waters and Places, describes artificial elongation and molding of the heads of infants and says that in the land of the Macrocephalics, those persons endowed with long heads are classed as belonging to the noblest race. Certain tribes of Solomon Islanders likewise are proud of their artificially produced, gourd shaped heads.

was an Indian captive of the early Spaniards from Chicora (Carolina) in 1521. According to Francisco's fanciful tales, the people of Chicora were governed by a giant king called Datha whose enormous size was not natural but had been produced by softening and stretching his bones. Then again, an evident reference to the practice of moulding the feet of infants occurs in Radisson's account of his third journey in the Great Lakes region about 1658–1660, wherein he stated: "All knowes there one another by their march, for each hath his proper steps, some upon their toes, some on their heele, which is natural to them, for when they are infants the mother warpeth them to their mode."

Among the Songish Indians of British Columbia there is a saying: "Tous o'wana tans KsEtctca'ai,"—"as if no mother had made you look nice." Doctor Boas describes the Indian practice of "making the child look nice" as follows:

"As soon as it is born, the mother rubs it from the mouth toward the ears, so as to press the cheek-bones somewhat upward. The outer corners of the eyes are pulled outward that they may not become round, which is considered ill-looking. The calves of the legs are pressed backward and upward, the knees are tied together to prevent the feet from turning inward, the forehead is pressed down." Among the Nootka Indians, according to the same authority: "Immediately after birth, the eyebrows of the babe are pressed upward, its belly is pressed forward, and the calves of the legs are squeezed from the ankles upward. All these manipulations are believed to improve the appearance of the child. It is believed that the pressing of the eyebrows will give them the peculiar shape that may be noticed in all carvings of the Indians of the North Pacific Coast. The squeezing of the legs is intended to produce slim ankles" (Chamberlain). Even today, the Indians of Oklahoma direct that to shape a baby's head round, press its forehead with the right hand, the back of the head with the left three times a day for four weeks. An infant's nose, they say, may be shaped by pinching it gently several times a day. The so-called Flat head tribes of Vancouver Island and the Chinook,

Nootka and Salishan tribes of the Columbia River country deformed the heads of their infants by strapping them between two boards which met at the top, or else compressed them with pads causing the forehead to slope sharply back from the eyebrows (Darby, 1933). The Coshuttas (Kaosoti) lived about 50 miles up the Trinity River in Texas. Flattening of the head among them was not a religious procedure but rather done for the cosmetic effect. The Coshutta's technique was as follows: The infant, after ten days, was taken from the mother's breast and strapped on a board as a cradle. A small concavity at one end of the board served to hold a lump of wet clay in which the infant's head was imbedded, the infant lying on its back. Another lump of clay was strapped or tightly bandaged over the infant's forehead, the bandage being gradually tightened as the soft skull yielded to the pressure. In 1820, head-flattening was practiced by the Koosoti and a few of the Bidai, but not by the Carancahuas, Coker, Cujanos or Tonkaways (Dyer, 1916). De Soto observed this practice among the Indians of Florida; it was reported among the Choctaws, Chickasaws, the Waksaws of North Carolina, the Creeks, the Muskogees around the Gulf of Mexico, the Natchez, Catawbas, Attacapas of the west bank of the Mississippi, and among the Solkeeks in Northern Louisiana. In Central America the custom was found among the Caribs and upon images on ancient Mayan remains, as well as among the ancient Zapoteks, predecessors of the Aztecs of Central America. It was interdicted by a Synod of 1585 under pain of heavy punishment among the Peruvians (Ploss, 1876). Another reason for this treatment of the skull may have been based upon primitive desire to aid in the closure of the sutures, gathering in the bones of the head, as the Finns expressed it. Among the Lummi Indians of Northwest Washington the head is flattened as a sign of rank (Stern, 1924). The Chinooks, of Oregon, also compressed the head to shape it in a peculiar way, but this was not permitted to the children of their slaves. This practice is also known in other parts of the world. Other forms of binding were practiced; the Kutchins bandaged their feet in order to keep them small and the Flatheads and Pend d'Oreilles bandaged the waist and legs of

infants with a view to producing broad-shouldered, small-waisted and straight-limbed adults. The Pericuis and Guaicuris fixed sand bags to the chest of their male infants to prevent the breasts from growing like a woman's, which they considered a deformity. Skull deformation was not observed among those Caribs who bound the legs of their children as described by Humboldt. It was common, however, among the Caribs of Parima and French Guiana. In some osteological collections, skulls supposed to be those of Caribs of the island of St. Vincent are in fact skulls shaped by having them pressed between planks. They have belonged to Zambos (black Caribs) who are descended from Negroes and true Caribs. The barbarous habit of flattening the forehead is practiced by several nations, of people not of the same race. Nothing is more vague than the conclusion that some degree of conformity in customs and manners proves identity of origin. Walckener reported head flattening among the Tapoyranas of Guiana and the Solkeeks of Upper Louisiana. The Indians of Cumana, says Gomara, press down the heads of young infants tightly between cushions stuffed with cotton, for the purpose of giving width to their faces, which they regard as beauty. The young girls wear very tight bandages round their knees in order to give thickness to the thighs and calves of the legs (Humboldt, v. 3, p. 85).

Humboldt in his travels met some Caribs of Panapana near the port of Encaramada in Northern South America who were of pure race but, unlike their ancestors from the West Indies whose skulls preserved in the collections of Europe showed evidences of head flattening, these had well rounded foreheads. These women, who were very tall and disgusting from their want of cleanliness carried their infants on their backs. The thighs and legs of the infants were bound at certain distances by broad strips of cotton cloth, and the flesh, strongly compressed beneath the ligatures, was swelled in the interstices. The Caribs attached great importance to certain configurations of the body and a mother would be accused of culpable indifference toward her children if she did not employ artificial

means to shape the calf of the leg after the fashion of the country.

"We observed with pain the torments which the Carib mothers inflict on their infants for the purpose not only of enlarging the calf of the leg but also of raising the flesh in alternate stripes from the ankle to the top of the thigh. Narrow ligatures consisting of bands of leather or of woven cotton, are fixed two or three inches apart from each other and being tightened more and more the muscles between the bands become swollen. I observed, however, with surprise that the manner in which these children are bound, and which seems to obstruct the circulation of the blood, does not operate injuriously on their muscular movements. There is no race of men more robust and swifter in running than the Caribs" (Humboldt, v. 3, p. 84).

In examining the skeletal remains exhibited by the Peruvian collection, Dr. Corlett noted especially the evident high rate of infant mortality. Many of these deaths he thought were due to negligence, improper diet and undue exposure to the elements. Many of the diseases more peculiar to childhood would leave no mark upon the skeleton. Hydrocephalus is suggested by the shape of several ancient infant mummies in South America. In common with the rest of the New World, rickets does not seem to have been present in the Peruvian region, but a disease of which we do have evidence in the bony frame of the individuals, resembling rickets in its tendency to attack the young, is called symmetrical osteoporosis. A theory has been offered that a diet restricted chiefly to corn and lacking such elements as calcium, phosphorus and vitamins C and D, as would be found among a strictly agricultural population, was responsible for the disease. The osseous signs include the formation of honeycomb-like patches in the skull with spongy thickening of the bone layer. The disease is labeled "symmetrical," because it presents itself on both sides of the skull and usually proceeds from the roof of the orbit to the parietal and occipital bones. The individual having survived to adult life, the skull shows a transformation of the outer spongy bone to more compact tissue with sieve-like

evidence of the ravages of the disease (Corlett, 1935). This condition is described by Roy L. Moodie also as occurring among the Ancient Egyptians, and he classes it, too, as a nutritional disturbance, evidently having its inception in infancy or early childhood (Moodie, 1923).

In the "Neanderthal Man," which in reality consists of some portions of a skeleton of extinct species of man found in a cave in the valley of the Neander River in the Rhine Province of Prussia, Rudolph Virchow thought that the condition of the bones indicated rickets. If so this would be the oldest evidence of rickets in man; but Schwalbe restudied the question and decided that there was no evidence of malnutrition and his conclusions are widely accepted (Moodie, 1923). Ruffer, in a study of skeletons of various dates (712 to 550 B.C., and 100 B.C. to 300 A.D.), found two bones showing deformities due to rickets (Moodie, 1923), and the disease was found in the skeleton of an ape of ancient Egypt, yet on the whole, early skeletal remains do not often show evidence of rickets in America or abroad. Even among the skeletal remains of the mound builders of ancient times in the Mississippi Valley there is no definite evidence of rickets. However, in the United States Army Medical Museum there is a deformed radius of a youth aged about 19, which is labeled "probably rickets." The osteologic collection of the United States Medical Museum also contains a skull of a pre-Columbian boy of Peru, age about 5 years, which is typically rachitis, labeled "rickets and probably syphilis" (Foote, 1927). In addition to rickets it is curious to note that there are little clay images found in the graves of children in Tennessee, Arkansas and Missouri which are faithful representations of persons afflicted with Potts disease, and many of the water bottles from the stone graves of Tennessee and from mounds of Missouri represent women with hunchbacks; but since tuberculosis of the spine is seldom indicated in skeletal remains, it is possible that the clay images represent other spinal deformities (Moodie, 1923). Club foot occurred in Peru as shown in Inca huacos, and harelip has been noted in Inca skulls. Thus we see that very few traces of

diseases in prehistoric infants have left their imprint upon the records of time.

There is considerably more information about the medicine of the American aboriginals from the time of the coming of the white man on to the present. Eric Stone, William T. Corlett and others have written extensively on the subject. But no one, so far as I know, has laid any particular emphasis upon the care of the child. It is only from fragmentary passages hit upon by chance reading here and there that we are enabled to piece together the manners and customs of the Indians relevant to the infant and child in health and in disease. The ubiquitous Dr. Benjamin Rush, whom we shall meet often in various fields of our study of the early American Pediatric literature, in writing "An Inquiry into the Natural History of Medicine Among the Indians of North America" which he read before the American Philosophical Society of Philadelphia on the fourth of February, 1774, revealed some of his pediatric beliefs: "I. Of the birth and treatment of their children.

"Much of the future health of the body depends upon its original stamina. A child born of healthy parents always brings into the world a system formed by Nature to resist the causes of diseases. The treatment of children among the Indians tends to secure this hereditary firmness of constitution. Their first food is their mother's milk. To harden them against the action of heat and cold (the natural enemies of health and life among the Indians) they are plunged every day into cold water.[2] In order to facilitate their being moved from place to place, and at the same time to preserve their shape, they are tied to a board, where they lie on their backs for six, ten or eighteen months. A child generally sucks its mother till it is two years old, and sometimes longer. It is easy to conceive how much vigor their bodies must acquire from this simple, but wholesome nourishment. The appetite we sometimes observe in children for flesh is altogether artificial. The peculiar irritability of the system in infancy forbids stimulating

[2]Galen censured the Germans who ducked their newborns into ice cold water as an ordeal to eliminate weaklings.

aliment of all kinds. Nature never calls for animal food, 'till she has provided the child with those teeth which are necessary to divide it. I shall not undertake to determine how far the wholesome quality of the mother's milk is increased by her refusing the embrace of her husband during the time of giving suck" (Rush, 1805).

The hardening process of the child, instituted in the new born with its first bath, is frequently mentioned by many observers, and infanticide among the early primitive tribes was not uncommon. Deformed children were not often seen among the Indians, and the inference has been that the weak or congenitally defective were destroyed at birth or soon after. Among the Indians on the Amazon River the child was exposed to a test for the right to survival; all infants immediately after birth were submerged in a stream, but the deformed children were never pulled out again. The primitives everywhere had a horror of malformations (Miller, 1928). Cieza referred rather credulously, though in detail, to the monsters among the Cuzco Indians born from sexual intercourse with monkeys in the case of men, and with dogs in the case of women (Moll, 1944). Among the Santee, Crees, Muscogees and Assiniboins, children deformed or blemished after delivery were killed or received a special name for their peculiar feature. If a deformed child was born to a mother of the Mosquito group of Central America, it would receive a nickname that would be a disgrace to the family. During childbirth the woman remained in seclusion in a hut in the forest where it was easy for her to dispose of a deformed child, and it is believed that this was usually done to avoid such disgrace. Alexander Von Humboldt (Personal Narrative of Travels to the Equinoctial Regions of America, During the Years 1799–1804) saw no person among the Chaymas, Caribs, Muyscas, and Mexican and Peruvian Indians during the course of five years who had any natural deformity. Bodily deformities and deviations from nature, he declared, are very rare among certain races of men, especially those who have the epidermis highly colored. He reasoned that among primitives a deformed or very unhealthy woman did not succeed in acquiring a mate and died childless; with copper

colored men, as with whites, he thought, luxury and effeminacy weakened the physical constitution and therefore deformities were more common at Cuzco and Tenochtitlan where Bernal Diaz saw dwarfs and hunchbacks waiting at the tables of Montezuma. In a village of 2000 pure blooded Indians and halfbreeds in the eastern border of Peru, an observer had never seen a deformity in Indian children, nor had Padre Ignacio, the parish priest, during a residence of nine years (Corlett, 1935). Samuel R. Lothrop, connected with the mission among the Valigan and Ona Indians, believes that abortion and the abandonment of children among these South American tribes was of no infrequent occurrence. Thomas Whiffen, writing of the Indian tribes of the Northwest Amazon region found that Bush-deer, as a food, was tabooed to all women after marriage among the Kuretu-language group, because they believed it would make the infant deformed, and no greater disgrace could befall a Kuretu woman than to be the mother of a deformed child. Even twins, which are not in good form among them, can be condoned, but a deformed child cannot be disposed of without exciting gossip. The accidental death of one of the twins is regulated by good usage. Thus the second child, if of the same sex, is considered an interloper with no right to live; if a boy and a girl are born, the girl is sacrificed, regardless of which is born first. Since birth for the most part takes place in the bush with but one trusted female attendant, it is not difficult to cleanse hands thus steeped in human blood (Corlett, 1935). Las Casas tells of a Cuban Indian who had quintuplets in the sixteenth century. What feelings such an event must have stirred up in the primitive minds! Twins usually were regarded as uncanny and were rather feared as possessing occult power. With some Oregon tribes and other West Coast tribes they once were regarded as abnormal and one or both were killed. When twins were born among the Indians of Guiana, in South America, false notions of propriety and family honor required that one of them should be destroyed. "To bring twins into the world," said the Indians, "is to be exposed to public scorn; it is to resemble rats, oppossums and the vilest of animals" (Humboldt, v. 2, p. 248).

Nay, more, they affirmed that "two children born at the same time could not belong to the same father." This was an axiom of physiology among the Sulives, a tribe inhabiting the equinoxial regions of South America. To avoid the disturbance of conjugal tranquility the old female relations of the mother took care that when twins were born, one of them should disappear. If a new-born infant, though not a twin, had any physical deformity, the father instantly put it to death. They would have none but robust and well made children, for deformities indicated to them some influence of the evil spirit Ioloquiamo, or the bird Tikitiki, the enemy of the human race. Sometimes children of a feeble constitution had to undergo the same fate. While the action would be disavowed by the perpetrator, it was not considered criminal. These primitives killed their children to avoid ridicule or avoid inconvenience. These acts of cruelty were less frequent, however, than these accounts would lead us to believe.

The Paraguayan Indians defended their right to kill the newly born whenever they willed it. They claimed the right to the life which they had given. In the Chaco of Paraguay, the child was simply burned, there were no rites and no mourning beyond the death-wail of the mother. When a mother of a young infant died among the more backward Amazon natives, they threw such a child to wild dogs or buried it alive with its mother. The Malamutes, a tribe of Esquimos inhabiting the region of Norton Sound, prize boy babies, but frequently kill the girls, taking them out into the wilderness, stuffing grass into their mouths and abandoning them; yet children were highly esteemed, and the barren woman was a reproach among her people. Simpson affirmed that the Loncheux mothers originally had a custom of casting away their female children, but later it was done only by the Mountain Indians. Abortion and infanticide were not uncommon among the Haidah Indians of British Columbia, the Chinooks and others, especially if illegitimate. With the Pend d'Oreilles, another of these Columbian tribes, it was not uncommon for them to bury alive the very old and the very young, because they said, "These cannot take care of themselves, and we cannot

take care of them, and they had better die" (Bancroft, 1882). The Pericuis and Guacuris also have been known to abandon or kill their children when food was scarce. There was a tradition among the Indians of South America that a tribe of Amazons inhabited the Rio Cuchivero in Guiana. They were called Aiklambenanos which signifies "women living alone." Once a year they admitted men of the neighboring nation of Voklaros into their society and sent them back with presents. All the male children born in this horde of children were killed in their infancy (Humboldt, v. 2, p. 401). Several tribes in Guatemala were strictly opposed to marriage outside the tribe and would destroy the progeny left by a stranger. According to Hugo Reid, who derived his information from traditions, the natives of the region of Los Angeles were greatly terrified at the first sight of the Spaniards. Women used by the soldiers were obliged to undergo a long purification and for a long time every child with white blood in its veins was strangled (Bancroft, v. 18, p. 180). Among the Nishinain of California, custom provided that the relatives destroy the very young infant on the death of the mother. Some of the peoples of Central America also practiced such customs. When a mother died while suckling her infant, the living child was placed at her breast and buried with her in order that in her future state she might continue to nourish it with her milk. Among the Chiloctin Indians of Canada, the death of a mother during delivery led to burying the child in its cradle with the mother. In the Paraguayan Chaco, infants were murdered if either father or mother died at time of birth (Miller, 1928). Sacrificial burial of wives and children was also widely practiced. Blair Niles saw the mummy of an old man in an Ancient Peruvian cemetery with which had been buried five children, three of them babies, sacrificed evidently in honor of the exalted rank of the dead man. Samuel Lee observed that the Indian women in New England often poisoned themselves and their children if their husbands would not "owne" them (Publications of the Colonial Society of Massachusetts, 1913). During the siege of Mexico City by Cortes in 1521, it is stated by Indian authorities that among the besieged Aztecs

many a mother, in her agony, devoured the offspring which she no longer had the means of supporting due to the famished condition of the doomed inhabitants. This is recorded of more than one siege in history.

The initial cold bath was considered by some to be also a weeding out process, for only the hardiest babies were presumed able to survive the rigors of the cold bath. In writing home to the Society of Free Traders in England in 1683, William Penn also mentioned this custom: "I will begin with the children. So soon as they are born, they wash them in water, and while very young, and in cold weather to chuse, they plunge them in the rivers to harden and embolden them. Having wrapt them in a clout, they lay them on a straight thin board, a little more than the length and breadth of the child, and swaddle it fast upon the board to make it straight; wherefore all Indians have flat heads; and thus they carry them at their backs. The children will go very young, at nine months commonly; they wear only a small clout round their waist till they are big" (Meyers, 1912). That the Aborigines were as kindly concerned about their young as any others of the human race is shown when he writes further: "In sickness impatient to be cured, and for it they give anything, especially for their children, to whom they are extremely natural." As a rule, both parents were alike equally devoted to their children and bestowed upon them the fullest expression of affection and solicitude. Lewis and Clarke stated that Indian children were never flogged as it was thought to break their spirit.

While two early American travellers were in the house of some Indians, a little naked child fell from its mother's lap and received a cut in its head, whereupon all who sat around that fire and belonged to that household, husband, wife, young and old, began to cry and scream more than the child, and as if they themselves had suffered serious injury (Dankers, 1867). Pastorius, writing of the same tribes in 1697, stated: "They love their children passionately. They bind them on shingles as soon as they are born. When they cry the mothers move them rapidly to and fro, and so quiet them, and although

they are still young they plunge them into the warm rivers that they may so much sooner grow strong. In their infancy they are made to catch fish with their hooks; afterwards, when they grow stronger, they train themselves in the hunt" (Meyers, 1912). He also mentioned that they frequently greased their children with a fat of bears and other animals, so as to make their skin dark, he thought. An even earlier description of the swaddling of Indian infants was written by Peter Lindestroem in 1653:

"As soon as the children are born to the world the savage female takes the child and binds it to a piece of board, which is just as long as the child, with three long braids, and does not place the smallest particle of clothing under or above the child. The first braid she wraps over the forehead and around the board, the second braid, right over the arms and the board, the third braid right over the knees and the board, tying it right strongly to the board. The savage female never loosens it from the board, while it is quite young, except when she must do so for sanitary reasons. Nor do we ever hear their children cry or whine like the Christian children, but they are very patient. But when some illness attacks them then they lament so miserably and quietly with a soft sound. However, they show themselves very tender towards their children. When now the children finally get out of this swaddle, they carry their children with them on their backs just like the gypsies do" (Johnson, 1925).

Young Indian mothers sang to their babies to put them to sleep and had many stories which they told to children a little older with the same purpose. The tales were usually about small animals; about the mice, the little squirrels or the chipmunks.

The Indian infant's education began at an early age, its mother teaching it first to keep quiet. Crying babies were hushed, or, if they did not cease their noise, were taken out of the lodge and off into the brush where their screams would not annoy anyone. If older children were talking and a tiny child entered the lodge and began to talk to its mother, she held up

her finger warningly and it ceased to talk or else whispered its wants to her. Thus the first lesson that the child learned was one of self control; self effacement in the presence of its elders. It remembered this all through life.

Children, as well as women, were universally exchanged, adopted, bartered or sold into slavery, frequently for trivial amounts. Among the Utahs, great numbers of children were sold to the Navajos for blankets. An act was passed by Utah in 1852 legalizing slavery, which set forth that from time immemorial slavery had been a customary traffic among the Indians; that it was a common practice among them to gamble away their wives and children into slavery, to sell them into slavery to other nations, and that slaves thus obtained were barbarously treated by their masters; that they were packed from place to place on mules; that these unfortunate human beings were staked out to grass and roots like cattle, their limbs mutilated and swollen from being bound with thongs; that they were frozen, starved, and killed by their inhuman owners; that families and tribes living at peace would steal each other's wives and children and sell them as slaves. In view of these abuses it was made lawful for a probate judge, or selectmen, to bind out native captive women and children to suitable white persons for a term not to exceed twenty years (Bancroft, 1882). The Comanches, too, were notorious for their traffic in children. They not only stole children from other tribes and sold them, but carried on a most unnatural traffic in their own offspring.

The care of the newborn among the early New England Indians was described by the observant Rev. Lee as follows: "They wash them and put up their heeles to their nates and tye ym downe to a board and tis thought is ye cause they never are bursten; and when fowle wipe ym with mosse and tye them up againe and carry ym at yr backs" (Publications of the Colonial Society of Massachusetts, 1913).

George J. Engleman, whose book on Labor among Primitive People is a prerequisite in the study of the mother and child among the American Indians, gives a very full account

of the care of the new born. From an "Early History of Virginia" the initial bath and swaddling of the baby is described as follows: "The manner in which they treat their young children is very strange, for instead of keeping them warm at their first entry into the world, and wrapping them up in I don't know how many cloths, according to our fond custom, the first thing they do is to dip the child over head and ears in cold water, and then bind it naked to a convenient board, having a hole fitly placed for evacuation, but they always put cotton, wool, or other soft things for the body to rest on between the child and the board. In this posture they keep it several months, till the bones begin to harden, the joints to knit, and the limbs grow strong. Then they loosen it from the board, and suffer it to crawl about, except when they are feeding or playing with it. While the child is thus on the board, they either lay it flat on its back, set it leaning on one end, or hang it up by a string fastened to the upper end of the board, the child and board being all the while carried about together. As our women undress their children to clean and wash their linen, so they do theirs to wash and grease them. The method the women have of carrying their children after they are suffered to crawl about is very particular. They carry them at their backs in summer, taking one leg of the child under their arm and the counterarm of the child in their hand over the shoulder, the other leg hangs down, and the child all the while holding fast with its other hand. But in winter they carry them in the hollow of their match-coat at their back, leaving nothing but the child's head out." This is reminiscent of the picture of the Esquimo squaw and child, with which everyone is so familiar. Simpson stated that the Esquimo women carry their infants between their reindeer-skin jackets and their naked backs (Bancroft, 1882). This would, of course, afford the child the warmth of its mother's body, a most effective means of doing so. Many of the other North American mothers also carried their children about in somewhat similar fashion, either bound in a blanket or slung in some kind of basket bag. The child was tucked away in an equally peculiar manner by some of the Polar tribes of Russia; until it begins to crawl it is placed in a

fur sack and carried by a strap about the mother's forehead. Later it is sewn up in a fur garment of one piece; for the sake of cleanliness a doorway is left in the posterior portion which is opened as necessity demands, but the garment is not once removed or changed until outgrown by the child. With such a practice in vogue one can readily believe that lice and typhus were common concomitants in afflicting these poor youngsters. Indeed the universal prevalence of the body louse among the American Indians leads me to believe that many of the devastating epidemics of measles that killed off so many of the natives of all ages were in fact typhus fever.

In Chile, the Araucanian Indians wrapped their children after birth in a woolen cloth, and a box bedded with the same material formed their crib. This the mother slung over her back. Little care was given to the rearing of children by these Indians and the death rate was high (Corlett, 1935). Old people of the Cheyennes told Grinnell that in earlier days when the tribe lived in lodges and moved about over the prairie, a large proportion of the children born in winter died. It can readily be understood that while living this nomadic life, it might have been very difficult to protect newborn children from the cold.

Father De Smet, a Jesuit missionary who travelled among the Western Indians of the United States in the 1840's gave the following picture of the care of the child. "In general great attention is paid to the physical development of the children and they are prepared from their tenderest infancy for the hardest kind of life. As soon as the child is born, in whatever season, regardless of the severest cold or the greatest heat they at once plunge it several times into water. Then, suitably wrapped up it is placed and entrusted in the hands of some other nurse than its mother. After the first week, its parents take it back and it is put into the cradle or berceau, a machine that deserves to be patented and which the little individual does not leave until he is able to walk. This is the simple construction of it; a small hide covered board about a foot longer than its little occupant. The child is placed upon it and tied tightly with bandages beginning with the legs and covering

it to the shoulders; they are made tightest about the loins and the pit of the stomach, in order to force out the chest as much as possible. Neatly arranged in this style, the little party occupies a place in the lodge out of harm's way. When the weather is fine, he is set in the doorway of the lodge. If the nurse goes away she hangs the cradle to the branch of a tree where the baby warms himself tranquilly in the sun, or on hot days is in the shade, and is in no danger from dogs, wolves or snakes which are often plentiful in the neighborhood. When they travel, the cradle is hung from the saddlebow where it is out of the rider's way and offers no danger to the little prisoner."

In 1863, Dr. W. P. Melandy, describing the Indian obstetrical procedure as observed at the Round Valley Reservation of California, reported that the new born baby seldom was washed, but after being wiped he was wrapped in a blanket then placed in a specially formed basket in which he was carried about on his mother's back for five or six months. The Indians of Southern California were aware that medicine given to a mother would affect her suckling babe. They bathed their infants three times a day for five or six months, beginning when it was five or six days old. When it was aged one month, it was placed in la cuna, or cradle, slung to the mother's back and kept there for two or three years, being allowed, when able, to walk or run about for a short time each day. In consequence of this hygienic custom, a stooped or bowlegged Indian seldom was seen. Chorea was never heard of among them, he averred (Bard, 1894). However, many Indians, especially of the more Northern families, were described as being bow-legged, but this usually was attributed to the manner of squatting while riding in their canoes. An anonymous writer who lived among the Indians of the central plains just after the time of the Civil War gave the following description of the Indians: "The love of an Indian mother for her child is made plain to us by the care and labor which she often expends upon the cradle: The choicest production of her skill in grass and woolen weaving, the neatest needlework, and the richest bead embroidery that she can devise and bestow are

lavished upon the quaint-looking cribs in which the savage mothers nurse and carry their little ones around. The governing principle of a papoose cradle is an unyielding board upon which the baby can be firmly lashed at full length on its back. This board is usually covered by softly dressed buckskin, with flaps and pouches in which to envelop the baby; other tribes, not rich or fortunate enough to procure this material, have recourse to a neat combination of shrub-wood poles, reed splints, grass matting and the soft and fragrant ribbons of the bass or linden tree bark. Sweet grass is here used as a bed for the youngster's tender back, or else clean, dry moss plucked from the bended limbs of the swamp firs; then, with buckskin thongs or cords of plaited grass the baby is bound down tight and secure.

"Indian babies, as a rule, are not kept in their cradles more than twenty to twenty-four consecutive hours at any one time; they are usually unlimbered for an hour or two every day, and allowed to roll and tumble at will on a blanket, or in the grass or sand if the sun shines warm and bright. The moment a baby begins to fret or whimper the mother claps it back into its cradle, where it rests with emphasis, for it can there move nothing save its head.

"When the papoose is put away in its cradle the mother has little or no more concern with it other than to keep within sight or hearing. If she is engaged about the wigwam or in the village, she stands it up in the lodge corner, or hangs it to some convenient tree, taking it down at irregular intervals to nurse. When she retires at night, the baby is brought and suspended at some point within easy reaching; if the baby is ill, it is kept at her side, or she sits up all night with it in the most orthodox fashion. When the women travel, the cradles, with the babies therein are slung upon the mothers' backs, and carried, no matter how far, how rough the road, or how dismal the weather" (Wild Babies, Harper's New Monthly Mag., 1878). An interesting and familiar device was used by some of the Indians living about Vancouver Sound. Infants, properly bound up with the necessary apparatus for head-flattening, were tied to the cradle or to a piece of bark and hung by a

cord to the end of a springy pole kept in motion by a string attached to the mother's great toe (Bancroft, 1882).

The Jivaro mothers along the Amazon and the Camacans in Brazil always carried the child on their arms. Among the extinct Incas of Peru the mothers were forbidden to carry their infants out of fear that this would soften them, the little child was set in a hole in the ground or placed in a cradle, but never allowed in the arms of its mother; even the prince royal was never carried about in arms. Among some of the Indians of California as soon as the child saw the light of day, it never got any other bedding than the hard ground, or the mother placed it in a turtle shell which was harder yet, and in this shell carried it around. However, in order not to deprive it of the freedom of its limbs, when the mother went out upon the fields she turned the child over to an old woman who often left the poor creature without any care for more than ten hours. When the child was a few months old the mother placed it, naked, astride her shoulders with its legs hanging down in front of her; thus the child learned to ride before it could even stand on its legs (Ploss, 1876).

Richard Matthews Hallett, in a delightful and adventurous book called "Michael Beam," a story of the early settlements of the middle west, described a cradle-board or baby frame as being perhaps two feet long, with a curved piece of board at one end to hold the child's feet, and a hoop at right angles above the other end. This hoop would serve as a support for a blanket in winter or a cloth in summer. A sort of spider web of red cords was hung on the hoop to catch evils that might threaten the child. The board was painted in brilliant reds and blues. This cradle was lovingly prepared by the expectant mother long before her baby was born.

The expectant Indian mother also gathered moss of the sort that grew in cranberry marshes; she dried it over a fire to destroy any insects, and then rubbed and pulled it apart until it was soft and light. When the weather grew cold, she would wrap her baby's feet in rabbit-skin with the hair inside, or in the soft down of cat-tails. The moss would be used to cleanse the infant. According to F. W. Hodge (1907), among some

tribes, notably those of the North American plains, in anticipation of the new arrival, the father prepares the wooden frame of the cradle which is to be its portable bed until it is able to walk. The body of the cradle, with its ornamentation of bead or quill design, fringes and bangles, is made either by the grandmother or by some woman noted in the tribe for her superior skill. One cradle often was used for successive infants in the same family.

The parts of the cradle as enumerated by Hodge, are the body, the bed, the covering, the pillow and other appliances for the head, including those for head flattening, the lashing, the foot rest, the bow, the awning, the devices for suspension, and the trinkets and amulets such as dewclaws, serving for rattles and moving attractions as well as for keeping away evil spirits. Cradles differed in form, technic and decoration, differing with the demands of the indigenous culture and environment. The Canadian and Alaskan Indians used birch bark while the Pacific Coast Indians were borne in wooden cradles or baskets of woven bark on beds of the shredded bast. In the Southwest, the baby-board had a cover of matting. The bark cradles of the interior of Alaska and the Mackenzie River basin were made of a single piece of birch or other bark bent into the form of a trough, with a hood and tastefully adorned with quill work. The bed was of soft fur, the lashing of babiche, thongs of skin, particularly of eel skin. They were carried on the mother's back by means of a forehead band.

Skin cradles were adapted in the areas of the buffalo and other great mammals. The hide, with the hair on, was rolled up, instead of the bark and in much the same way, to hold the infant; when composed of hide only they were seldom decorated. On the plains, cradles were made of dressed skins lashed to a lattice of flat sticks, especially among the Kiowa, Comanche and others. Over the face was bent a flat bow adorned with pendants or amulets. The whole upper surface of the hide was a field of beadwork, quill work or other decoration, in which symbolic and heraldic decorations were wrought. The frame was supported and carried on the mother's back

or swung from the pummel of a saddle by means of bands attached to the lattice frame in the rear. Among some tribes, the upper ends of the frame projected upward and were decorated. Among the Iriquoian and Algonquian tribes of the East, a thin, rectangular board took the place of the lattice. It was frequently carved and gorgeously painted, and had a projecting footrest. The bowl was also bent to a right angle and decorated. The infant, after swaddling, was laid upon the board and lashed fast by means of a long band. The tree for the Pawnee cradle-board was carefully selected, and the middle taken out so that the heart or life should be preserved, or else the child would die. Equal care was taken that the head of the cradle should follow the grain. The spots in the wildcat skin used for a cover symbolized the stars, the bow the sky and the crooked furrow cut thereon signified the lightning whose power was typified by the arrows tied to the bow. All the parts were symbolic.

On the North Pacific coast the infant was placed in a little box of cedar; the region furnished the material and the adz habit, acquired in canoe excavation, made the manufacture easy. Interesting peculiarities of these cradles are the method of suspending them horizontally, as in Siberia, the pads of shredded bark for head flattening, and the relaxation of the child's body in place of straight lacing. Decorative features were almost wanting. Closely allied to these dugout cradles and similar in arrangement of the parts were those found in contiguous areas made from the bast of cedar. In British Columbia the dugout cradle was beautifully copied in coiled work and decorated imbrications. Along the Pacific slope the basket cradle was common. The Salish developed such variety in basketry technic that mixed types of cradles were not surprising. The Yuman and Wichita tribes made hurdle cradles consisting of a number of rods or small canes or sticks arranged in a plane on an oblong hoop and held in place by lashing with cords or splints with the bed made up of shredded cottonwood bast. The Apache, Navaho and Pueblo tribes combined basket, hurdle and board cradle, the Navaho covering the framework

with drapery of the softest buckskin and loading it with ornaments. The ancient cliff dwellers used both the board and the hurdle forms. Here and there were tribes that placed their infants in network or wooden hammocks suspended by the ends. In these the true function of the cradle as a sleeping place was better fulfilled, other varieties serving rather for carrying.

Among the San Carlos Apache, at least, the cradle was made after the baby was born, to fit the body; later on a larger one was prepared. The infant was not placed into the cradle at once after its initial bath; a certain number of days elapsed and then it was done with appropriate ceremonies. When the mother was working about the home the infant was laid on a robe or mat and allowed free play of body and limbs. The final escape from the cradle was thus gradual, the process taking a year or more.

In many tribes scented herbs were placed in the bedding. Among the Yuma, difference was sometimes made in adorning boys' and girls' cradles, the former being much more costly. Some tribes made a new cradle for each child, but among the Pueblo tribes, particularly, the cradle was a sacred object, handed down in the family and the number of children it had carried was frequently shown by notches on the frame. It was believed its sale would result in the death of the child. If the infant died the cradle was either thrown away (Walapai and Tonto), broken up, burned or placed in the grave (Navaho and Apache) or buried with the corpse, laced up inside as in life (cliff dwellers, Kiowa).

Writing of the Winnebagoes, Surgeon David Day gave it as his opinion that the large percentage of deaths which occurred in the early periods of life among these Indians was abundantly accounted for by the Spartan treatment to which they were subjected in infancy. In this tribe, as soon as an infant was born it was laid on a board which previously had been covered with a few folds of blanket; then with a strip of cloth, 2 or 3 inches wide, it was as amply and securely bandaged from head to foot as an Egyptian mummy and then strapped to the board, care being taken always to include the arms,

which were extended upon the sides of the infant, and nothing left out of the bandage but its head. In this straightened position they spent the greater part of the first year of infancy, remaining at times for weeks without being taken from the board. The effect of this cradle with the heavy woolen bandages was to interfere with, if not entirely preclude, the healthy functions of the skin. The excrements of the child's body collected, excoriated the skin, and kept up a constant irritation. The motions of the limbs—the only voluntary exercise an infant could have and one so necessary to the development of its physical powers—having been entirely precluded, it soon became weak and enfeebled. But the most pernicious effect of strapping their infants upon these boards was exerted upon the brain. Being always laid upon their backs, with little or nothing between the hard board and the imperfectly ossified head, the continued pressure exerted by the weight of the head almost universally produced a displacement of the occipital bone inwards, causing trismus nascentium, paralysis, etc., and deranging the functions over which the cerebellum presides. They thought it a mark of comeliness to have the head perfectly flattened behind, and the Indian mothers showed much anxiety in this respect (The Scalpel, 1858). Daniel Drake also mentioned this flattening of the occiput of babies among the Natchez Indians of the interior valley.

Among the Sioux, Crows, Creeks and other North American Indians, as well as among some South American tribes, the mother plunged into the stream immediately after delivery, or if no running water was at hand, at least dipped the child in cold water as soon as it was born. The Apache Indians along the Colorado River, in place of the first bath, powdered the newborn baby with dry sand (Ploss, 1876). Among the Crows and Assiniboines, when a baby was born the umbilical cord was cut with a new butcher-knife, the stump was well greased, and the infant was then thrust into a laced sack made of blue cloth containing pulverized bull's manure or the inside bark of the cottonwood tree. This lining, with the child's discharges, was changed three or four times daily. When the stump of

the cord dropped off it was preserved in a beaded pouch and worn about the neck or waist as long as the person lived (Currier, 1891). The Mayan newborn had the cord cut with a stone, was bathed in warm water and buried to the neck under ashes to supply the lack of clothing (Moll, 1944). The birth of a son was celebrated with special rejoicings and extensive invitations issued for the feasts that took place on or about the day when the umbilical cord was to be cut, a ceremony which seems to have borne the same festive character as baptism among the Aztecs. Apparently the child remained with the navel string attached to it until a favorable day was selected by the Ahgih or astrologer for performing the ceremony of cutting it. The cord was laid upon an ear of maize to be cut off with a new knife and burned. The grains were removed from the cob and sown at the proper season, one half of the yield to be made into gruel and form the first food of the child aside from the mother's milk, the other half to be sent to the ahgih, after reserving a few grains for the child to sow with his own hands when he grew up, and make an offering thereof to his god.

Describing the birth of an infant to an Indian woman of the Illinois region in the days of the early American nation, Hallet pictured the old Indian woman in attendance as biting off the cord and cauterizing the stump with a live coal (Hallet). It is said of the Southern California Indians that as soon as a child was born, the mother herself tied the navel cord with a bit of buckskin string, severing it with a pair of sharp scissors prepared for the occasion, after which the end was burned with a coal of fire. The child was then thrown into the water; if it rose to the surface and cried, it was taken out and cared for. If it sank, there it remained and was not even awarded an Indian burial. A purification ceremony was then performed in which the mother and child participated. In the center of a hut a pit was filled with heated stones upon which herbs were placed, and the whole covered with earth, except a small aperture through which water was introduced. The mother and child stood over the pit wrapped in blankets and were soon in a violent perspiration. When they were exhausted

from the effect of the steam and heated air, they lay upon the ground and were covered with earth, after which they again took to the heated stones and steam. In some tribes the mother could hold no intercourse with her husband until the child was weaned. Among the Lummi Indians of Northwest Washington, when the child emerges from the womb, it is grasped by the right hand so that it will be right handed, or by both hands so that it will be ambidextrous. The navel cord is tied and cut and the baby bathed in luke-warm water, rubbed with dog-fish oil and wrapped with shredded cedar bark. The first feces of the baby is buried, not burned, to prevent the child's intestines from becoming weak (Stern, 1924). The Cheyennes and Arapahoes enveloped the child as soon as it was born in dry horse manure and did not wash it for several days. When a birth was about to take place among the Cheyennes, in more recent times, they had a bowl ready in which to wash the child, and a knife, in old times, of flint and later an arrowpoint was at hand to sever the umbilical cord, which was wrapped once about the finger and cut off short. The child was wrapped in a sheet or cloth, the inner surface of the legs being first dusted with powder from the prairie puffball, so that the tender skin should not chafe and the navel was dried with the same dust (Grinnell, 1923). Among the Hopi, ashes or sacred meal were rubbed on the newborn baby. On the first day of his arrival, the newborn Hopi's head was washed in soaproot suds and his diminutive body rubbed with the ashes, the latter it is alleged, to kill the hair, and his mother must also undergo the ceremonial head washing, which must be repeated on the fifth, tenth and fifteenth days with amole root, which is the only soap known to the Hopi.

Another writer states that the Hopi baby is first washed and dressed by its paternal grandmother or by one of her sisters. On the day of its birth she makes four marks with cornmeal on the four walls of the room. She erases one of these on the fifth, tenth, fifteenth and twentieth day of the child's life. On each of these days the baby and its mother have their heads washed with Yucca suds. On the twentieth day, which marks the end of the lying in period, the grandmother comes early,

bathes the baby and puts some cornmeal to its lips. She utters a prayer in which she requests that the child shall reach old age. Esther Goldfrank states that at the eastern Hopi pueblo of Cathiti, the pregnant woman keeps a perfect ear of corn in the house. After the baby is born she keeps a strong stick, the ear of corn and a light beside the baby until it is named, to ward off the evil spirits. The baby is washed in warm water, his head is washed and he is rubbed all over with oil. During the four-day lying in period a perfect ear of corn is kept beside the child and a small fire made from twigs kept burning. This may be used at any time to keep off the evil spirits. The Umpquas wrapped a newborn child in dirty rags and also put it away without washing. Among the primitive tribes that inhabit the region of Tierra del Fuego, the newly delivered mother, if near the sea or a river, plunged into the water for personal comfort, sometimes with the child in her arms. After this the child was placed between the breasts of the mother and covered with fine skins which had been especially prepared. This is in accord with Ratzel who says that the Fuegian woman has frequent and easy confinements, and to toughen the new born to withstand the rigors of the climate, when near the coast, it is immediately dipped into the sea (Corlett, 1935). The Ona mother of this same part of the world, in lieu of soap and when water is not at hand, covers and massages the child with white earth. Among some of the Eskimos, who are classified by ethnologists with the American Indians, the child is washed in snow, and is thus early in life, as in Tierra del Fuego, subjected to the most trying ordeals of nature. In spite of these hardships the race survives, although in many of the Northern climates it is gradually diminishing. Among the central Eskimos of Alaska the child is cleaned with a birdskin and clothed in a small gown of the same material. Then there are some Eskimos who lick their babies to clean them, lick them well over from head to foot as the cow does her calf (Chamberlain). The Iglurirmiut tribe of Eskimos swathe the child in the dried intestines of some animal. As soon as the Eskimo mother with her new born babe is able to get up and go out, usually in a few hours, the two are taken in charge

by an aged female angekok, who seems to have some particular mission to perform in such cases. She conducts them to some level spot on the ice, if near the sea, and begins a sort of march in circles on the ice, the mother following with the child on her back; this maneuver is kept up for some time, the old woman going through a number of performances, the nature of which could not be learned, and continually muttering something equally unintelligible. The next act is to wade through snow drifts, the aged angekok leading the way. We have been informed that it is customary for the mother to wade thus bare-legged. After a few days, or according to the fancy of the parents, an angekok, who by relationship or long acquaintance is a friend of the family, urinates into some vessel, and with the urine the mother washes the infant while all the gossips around pour forth their good wishes for the little one to become an active man, if a boy, or if a girl, the mother of many children. This ceremony, I believe, is never omitted, and is called "qoqsuiariva." Surely only the hardiest infant could survive such hardening procedures (Corlett, 1935).

Although the ancient Peruvians brought up their children with as little tenderness as possible, yet they never neglected continuously cleansing them. These inhabitants of the Inca empire washed the infant as soon as it arrived into the world, also with cold water, then it was bound in its wrappings; this was repeated every morning. If a mother wanted to give the baby a real treat, she took some water in her mouth and sprayed the child's whole body with it, except for the crown of the head which was never touched with water. The reason that they offered for this practice was that they accustomed the children in time to cold and discomfort and produced strong limbs (Ploss, 1876). Almost three months were allowed to go by before the arms were swaddled because they believed that the children might develop weak extremities from the wrappings. The Indians were not entirely unaware of the discomfort and unwholesome effects of uncleanliness. Grinnell reported that each morning the Cheyennes, men and boys, some of them little fellows just able to walk, came from the lodges and hurried down into the stream to plunge into it.

This early morning bath was a regular practice. It was done at most seasons of the year but some omitted the bath in winter when the streams were frozen, while others broke the ice and bathed in the cold water. This they considered good for health, to make them hardy and also to wash away sickness. The custom was peculiar to men; women did not do it. The practice was similar to that of the Blackfeet (Cheyenne Indians, 1923). Other tribes believed that boys should wash in swiftly running streams so that evil spirits would be washed away; but girls could wash in stagnant water. With the weaker sex, it did not seem to matter. The Cheyennes would not use water to drink that had stood over night, however. They said they wished to drink "living water."

Among the Caribs dwelling in equatorial regions of South and Central America, the children were not bound but were allowed complete freedom to toss about in their little hammocks. A Brazilian Indian who attended his wife at her delivery placed the child, without swaddling it, in a little bed of cotton suspended in the air. Potherius observed North American Indians who merely draped the newborn infant in a beaver skin no matter how raw the weather. But this was not true with all tribes. More often the Dacotas and the Sioux placed the baby, right after birth in beds filled with down and soft feathers from geese or swans; then they wrapped it warmly in a cloth for a short time, perhaps an hour or so. The cloth was removed, the child was washed and wrapped again in the cloth with a new band. The Thlinkets of Alaska, placed the child upon a board and wrapped it in moose skin when it was a few weeks old, while the Nootkas, further south, rolled the new born child in a mat among feathers.

The varieties of cradles are almost endless. We have the "hood" (sometimes the "boot") of the Eskimo; the birch-bark cradle (or hammock) of several of the northern tribes (as in Alaska, or Cape Breton); the "moss-bag" of the eastern Tinne, the use of which extended to the employees of the Hudson's Bay Company; the "tough-cradle" of the Bilqula; the Chinook cradle, with its apparatus for head-flattening; the trowel-shaped cradle for the tribes of the Oregon coast, the

wicker-cradle of the Hypas; the Klamath cradle of wicker and rushes; the Pomo cradle of willow rods and wicker-work, with rounded portion for the child to sit in; the Mohave cradle, with ladder-frame, having a bed of shredded bark for the child to lie upon; the Yaqui cradle of canes, with soft bosses for pillows; the Nez Perce cradle-board with buckskin sides, and the Sahaptian, Ute and Kootenay cradles which resemble it; the Moki cradle-frame of coarse wicker with an awning; the Navajo cradle, with wooden hood and awning of dressed buckskin; the rude Comanche cradle, made of lattice-work and leather; the shoe-shaped Sioux cradle, richly adorned with coloured bead-work; the Iroquois cradle (now somewhat modernized), with "the back carved in flowers and birds, and painted blue, red, green and yellow." Among the Araucanians of Chili we meet with a cradle which "seems to be nothing more than a short ladder, with crossbars" to which the child is lashed. In the tropical regions and in South America we find the habit of "carrying the children in the shawl or sash, and bedding them in the hammock" (Chamberlain).

Among many of the primitive tribes, placing the baby in its cradle had a very special significance. When the babies of the ancient Mexicans were given names, the last of all the rites which the midwife conducted on behalf of the child was to ceremoniously place the infant in its crib. Standing before it, with the baby among a procession of torch bearers, she made a prayer to Yoalticitl, the goddess of the cradle, commended the child to her care and protection and then turned to the cradle which she thus addressed: "You, Mother of the child, be kind to this little one and protect it so that no harm can come to it." She placed the child in the crib then, while the parents on their part prayed to Yoalticitl and spoke the same prayer. This ceremony was called "Tlalcoculaqulo."

Among the ancient Mexicans, Cihuacoatl or Quilaztli, was considered the first woman to have borne children, these being always twins. Zolotl was the divinity of twins and monstrosities; Ixtlilton or Tlaltecuin, a black deity, presided over the diseases of children. They believed that women who died in confinement wandered through the air afterward, possessing

the power of hypnotizing children (Flores, 1891). The Mayans relied on Zukuykak, or Ixmol, goddess of childhood to whom the month of Mol was consecrated, for the care and protection of children (Moll, 1944).

Among the villages along the Yukon River in Alaska a traveller reported that he saw little stools made of birch bark for small children. "The stool," he said, "has a stick of wood the purpose of which is to guard against any curvature of the little one's legs. The child sits comfortably upon a seat of moose skin and often would be carried around in such a chair upon the mother's back" (Ploss, 1876). Similarly the Kutchin women of the same region also carried their infants in a sort of bark saddle fastened to their backs, described by Whymper as "small chairs" and by Richardson as "seats of birch bark" (Bancroft, 1882).

The earliest Indians had no cows or substitute for the cow, for there were neither cows nor horses in America until the Spaniards brought them. An Indian slave of the Spaniards in 1521 related of the natives of Carolina that they had domesticated deer which generously furnished them with milk and cheese. His stories, however, were not wholly reliable. Occasionally among tribes on the buffalo plains of the middle west we find accounts of cases where a father himself has been obliged to care for and bring up an unweaned babe without a woman's help. "Young Woman," who was born in 1830, told G. B. Grinnell that she was so reared. Her mother having absconded, her father took the baby under his own care. He killed cows which had calves and cutting out the udders gave them to the child to suck. In the same way he killed female deer and antelope and let the child suck the udders. So he nourished her until he reached the Cheyenne camp where a woman took charge of the child. A like case was that of Arapaho Chief, a Northern Cheyenne (Grinnell, G. B., Cheyenne Indians, 1923). Usually the child was applied at once to the breast. Sometimes it was turned over to another matron to nurse until the mother's health was restored. The Cheyenne child was not allowed to nurse from its mother at first but some other woman who had a young child nursed it.

The medicine women for four days freed the mother's breasts from the early mammary secretion. During this time the mother was given doses of "mot si i yun," the milk medicine (Actaea Arguta), to induce a free flow of milk. Four days after birth the child might nurse from its mother. Among the Thlinkit Indians of Alaska, it was not considered safe to offer the baby the breast before all "impurities" had come away from the mother. These impurities, the source of later diseases, were to be removed by pressing and kneading the abdomen of the puerpera until vomiting was induced (Ploss, 1891). Among the same Indians, the new-born infant "is not given the breast until all the contents of its stomach (which are considered the cause of disease) are removed by vomiting, which is promoted by pressing the stomach," and among the Hare and Dog-rib Indians, "the infant is not allowed food until four days after birth, in order to accustom it to fasting in the next world." The Songish Indians do not give the child anything to eat on the first day. The Kolosh Indians, of Alaska, after ten to thirty months "accustom their children to the taste of a sea-animal," and among the Arctic Eskimos, Kane found "children, who could not yet speak, devouring with horrible greediness, great lumps of walrus fat and flesh." Klutschak tells us how, during a famine, the Eskimo of Hudson's Bay melted and boiled for the children the blood-soaked snow from the spot where a walrus had been killed and cut up (Chamberlain). One early writer about the Indians tells that the new born babe was immediately washed, whether it be summer or winter. The first nourishment given was the oil of some fish, or melted tallow of some beast; the infant was made to swallow it, and after that was given nothing but the mother's milk (Heagerty, 1928). Among Indians of California the old women who acted as midwives washed the child as soon as it was born and drank of the water; the unhappy infant was forced to take a draught of urine medicinally. The Hopi babies of more modern times (and of times gone by, I doubt not), are nursed as long as possible and are often fed piki bread and green corn very early. It was estimated by

Mary Robert Coolidge (The Rain-Makers, 1929, p. 64) that at least half of those born died of intestinal troubles.

As a rule the child continued to nurse as long as the mother's milk lasted or until another conception took place, a practice, as we have seen, lauded by Benjamin Rush as most salutory and commonly practiced in Europe among the gentility as well as the peasantry. John Jones, who in 1579 wrote the second book of pediatrics in the English language, remembered he was able to carry a stool for his good nurse when he wanted to suck (Radbill, 1938). Ancient authorities differed; some thought the best time for weaning was one year; others went up to three and four years as the optimum age. The Reverend Samuel Lee in 1687 stated that the Indians of New England suckled their young a year, commonly, and fed them with anything the child would eat, mentioning especially clams. If the mother died, the men gave the infants oysters to suck (Publ. of the Colonial Soc. of Mass., 1913). The Kanikars suckled the child for three to five years; the inhabitants of Sierra Leone often nursed the child until it could walk; the Alaskans, Dacotas, Sioux, Apaches and other native tribes, until the child was ten to thirty months of age. In other tribes the child was nursed until late in childhood, indeed even until puberty or longer (Ploss, 1891). Among the Arawak Indians of Peru, Dr. Farabee attributed the small size of the family to the fact that the women had children early and that the children nursed until they were three years old because of a lack of other proper nourishment. In 1807, Quandt reported that the Arawak women nurse their children until the next baby arrived, and then the grandmother, if one were around, took up the duty for some time longer. Appun often saw children standing alongside mother and grandmother calling upon the aid of both milk supplies. These women tried to maintain their flow of milk by suckling other young mammals. From his own observation Sir Robert Schomburk related the following concerning the Warrau Indians of British Guiana: "It is common for the children to be weaned only by the third or fourth year, so that often an older child is placidly standing by its mother and taking its customary nourishment,

while a younger one upon the arm of the mother is drinking at the second breast. But the most amusing sight was presented when a strapping lad who was first noticed up in the highest tree top of a Carico Papaya, suddenly clambered down laden with the fruit of this tree and ran to his mother in order to satisfy his thirst. But our amazement increased when we came upon four-footed brothers and sisters among the nurslings to which the mother just as obligingly, and with equal tenderness of glance and mien, offered her other breast, even though her own child was already sucking the first breast." Others have observed similar practices among the Makusis and Arekunas (Ploss, 1876). Of the Arekuna of Guiana, Schomburk tells us:

"They bring up children and monkeys together. The monkeys are members of the family, eat with the other members, are suckled by the women, and have great affection for their human nurses. Oftimes a woman is to be seen with a child and a monkey at the breast, the two nurslings quarreling" (Chamberlain). Among the Huichol Indians of Mexico the child nursed until the next pregnancy and in addition partook of food prepared for the mother. The most common mortality among young children was from diarrhea caused by eating unripe fruit. The mortality among the children of this tribe was high (Corlett, 1935). According to Lady Brown, the women of the Chucuaque tribe of Indians inhabiting the uncharted Island of San Blas nursed their children at the breast until they were six years old. This observer said she had seen many of them suckle in turn children of various ages (Corlett, 1935). The Fuegian women at the extreme southern end of South America customarily nursed their children until the age of three, and Dr. George E. Darby (1933) reported that most of the Indian women of British Columbia nursed their babies until they were fifteen months old. Among the extinct semi-civilized races of America we find a pronounced solicitude, manifested by definite rules and regulations for the whole manner and means by which the mothers conducted themselves in the process of nursing the infant. Among the ancient Incas of Peru, the women always nursed their own

infants. If a mother desired to offer her breast to the child, she laid over it; this occurred no oftener than three times a day: namely, morning, noon and night; except for these times, the child never received the breast, let it cry as much as it would, until it gradually became accustomed to this routine. This custom was observed by the women of the entire Inca realm; as the reason therefore they asserted that the children would become pale, apt to vomit and insatible with increasing age by too frequent nursing. No matter how proud or genteel a woman might be, she never entrusted her child to a wet-nurse unless she would be prevented from nursing because of illness. If the mother had sufficient milk for her child it got nothing else to eat or drink until it was weaned, for they believed that other foodstuffs given together with mother's milk would be injurious to the health of the child. As soon as a child could hold itself erect, it had to grasp its mother's breast the best way it could, without the mother even taking it upon her lap; if it desired the opposite breast, it would be held out to him and he had to reach for it himself without being taken up into the mother's arms. The test of a wet-nurse was to press out a drop of milk upon the nail, when if it did not run the milk was considered good. This test was a widely used one by the earliest European medical authorities and may have been introduced by the early Spanish arrivals. No food was given to the child the first day in order to create an appetite. It was suckled for three years, in some places much longer; during this time the mother adhered to a diet that would keep up the quality of the milk. Many abstained from sexual intercourse with their husbands for the same period to prevent the possibility of another child interfering with the proper nurture of the first one. A feast was given at the weaning of the child.

The women of the Aztecs always nursed their own babies; but when a mother was forced by illness to turn her child over to a wet-nurse, this substitute was chosen with the utmost care. Landa, about 1566, remarked that Mayan children nursed until three and four years of age, and that during the first couple of years they waxed "wonderfully pretty and

fat." This protective ancient custom of long continued nursing still postpones the problems of malnutrition until after the first infancy in some regions. In Paraguay, in rural areas, women nurse their children for two years supplementing the diet after six months with other foods. The Iriquois women believed that unless they nursed their own children they lost the qualities of a mother. They were amazed to learn that there were people who allowed strangers to nurse their children. However, if a mother died, wet-nurses were always acquired by every family; if necessary, even a grandmother, long past the age of fertility, could restore her milk supply and take the place of the mother. Among the Brazilian Indians, in addition to mother's milk the child's aliment included masticated meal. No mother among these would permit her child to be nursed by another woman; she would consider it disgraceful to give her baby to another woman to nurse, and so would only submit to such a procedure through dire necessity. The Caribs always nursed their own children, too, but as soon as the infants acquired a little strength, they began to feed them potatoes, bananas and other fruit.

To increase the flow of milk the ancient Aztecs advised the following: Where the milk flows with difficulty, take the plants chichiltic and xijhtontli, which shows acid slightly, the tohmigo xihuitl and crystal, ground in pulque and boiled. Let her drink it frequently. Afterwards, macerate the plant memya xiuhtontil in pulque and let her also drink that juice; let her enter the bath and there have another drink, made from corn. On leaving it, let her take the viscous water drawn from the grain" (Gates, 1933). These highly cultured people, upon delivery of the infant, carefully examined the child to be certain that it was fully developed, then, with much ceremony and many incantations, bathed the baby, scarified its genitals and clothed it. A Mexican stone (Tlacuiloltecpatl) (painted stone) possessed the virtue of increasing a new mother's milk similar to the European milk stone (galactites), though it is not the same thing, being a white, transparent stone spotted purple, scarlet and green (Loomis, 1944). The Hopi Indians, following the "doctrine of signatures" gave decoctions of milk

weed to mothers with insufficient breast milk. Onions were given to the Delaware Indian mother to produce milk, but care had to be taken not to use a quantity that would cause nausea for the child and the mother, for they evidently felt that the food could be transferred to the child through the mother's milk, a belief that was universal throughout all ages and localities, and now acknowledged to be only partially correct. The Actaea Arguta (Nutt.) was called Sweet Medicine or Sweet Root by the Cheyennes; motsi iun in their language. The roots and stems, after they had been dried, were pounded up to make an infusion to be given to the parturient to rid her of colostrum quickly. It was considered generally a good medicine for the blood. "Sweet Medicine," a Cheyenne hero, is said to have named this plant for himself, telling the people that this would help them to save and to bring up their children. Often it was boiled with Mertensia, which they called "blue medicine," (Mertensia siliata [Torr.]) and given to nursing mothers with an insufficient supply of milk for the purpose of increasing the flow. With these they also used the Oxytropis sp., a plant likewise useful for mothers whose milk did not seem to agree with the child. Lygodesmia Jiencea (Pursh.), called milk medicine was similarly utilized alone or in combination. The Cherokee Indians always nourished their infants with mother's milk unless it be brought up to be a witch, when no mother's milk is given for twenty-four days, only the liquid portion of corn hominy being given during this period of taboo; or in case the mother's lactation is deficient, which is only rarely the case, a decoction of the bark of the smooth sumac (Rhus Glabra) is drunk by the mother "to make the milk flow abundantly," and from then onward she nurses the children (Mooney, 1932). For dry breasts the Pawnees used infusions of Skeleton-weed (Lygodesmia jienceo) and there were many other natural remedies besides to which the mother striving to maintain or augment her supply of milk could turn. The Arawak women of South America, in order to maintain the flow of milk for as long as possible, placed little pigs and young puppies to suckle at their breasts. For the same purpose of prolonging the production of milk in the

breasts many other South American races followed a similar practice. The women of Makusis Indians of British Guiana maintained their milk for a long time and the child nursed until whatever age he pleased. If the family increased in the meantime then the grandmother assumed the duty of the mother in this respect. To these old women likewise fell the duty of suckling young mammals, opossums, monkeys, doe, etc., found accidentally. While the child was nursing from one breast these women were often found offering the opposite to these young animals with equal solicitude (Ploss, 1891). However, the dog was always the preferred adoptive of numerous tribes. Gabriel S. Theodat saw the Indian squaws of Canada allow young dogs to suck at their breasts many times. Indeed the dog played this role in many of the cultured regions of the world; the ancient ladies of Rome, the modern Neopolitans, Persians, etc., practiced a similar custom. Even as late as 1850, Dewees advocated the use of puppy dogs in this manner to increase the flow of milk, and I have known it to be advised in Philadelphia by midwives.

If the mother's milk failed completely it was not unusual among many of the American Indians for some other woman who happened to have an adequate supply to nurse the baby. Many a white infant in captivity was reared by an Indian foster mother, while other children in different parts of the country were often entrusted to friendly Indians in the neighborhood for wet nursing. In 1609, Philip III of Spain forbade Indian women with live babies to nurse Spanish infants (Moll, 1944). Spanish laws and regulations for the government of the Indies contained several provisions for safeguarding the health of Indian mothers and children, especially during lactation and pregnancy. It was early realized that the child of the wet-nurse (nodriza, Oma) needed special protection, and also the child for whom the nurses' services were secured. In 1537 the Spanish Crown forbade the employment of Indian mothers in Tucuman (Argentina) as wet-nurses unless the Indian infant was dead; this was extended to Paraguay and Rio de la Plata in 1609. Writing to the celebrated Dr. Nehemiah Grew in reply to some queries relating to the Indians about 1687,

the Rev. Samuel Lee stated that infant feeding was much at one with that of the English Colonists and that the English sometimes used Indian wet-nurses to good advantage (Publ. of the Colonial Soc. of Mass., 1913). Roger Williams noted that the Indian women nursed all their children themselves, yet if they were of high station or rich they maintained nurses to tend to the child. In the story of the trials and tribulations of Jonathon Dickinson, wife and infant who were captives of the early American Indians it is related that the wife of Cassakee, one of the Aborigines, having a young child sucking at her breast, gave it to another woman and would have Jonathon Dickinson's child, which its poor mother was very loth to suffer, but she would not be denied, took the child, and suckled it at her breast viewing it and feeling it from top to toe, and at length she returned it to its mother. Later, while attempting to make their way back to habitable civilization, for want of food of any sort, the hapless wife's milk was gone and her child was in great want. The Indians would now and then give it suck, but rarely to satisfy it. Though the mother had so little milk in her breast, yet when an Indian woman lately delivered had no milk herself, asked Jonathon Dickinson's wife to suckle her child also, which she consented to, it proved a means of her own child's receiving benefits, for the Indians would for that reason give her fish which helped increase her milk (Hazard, 1830). Even the father has been known in time of need to have resorted to nursing the baby. Franklin mentioned an old Chippewa who, on losing his wife at childbirth induced milk to flow in his breasts in sufficient amounts to be able to rear his child. Gynecomastia and the anomalous presence of milk in the male breast has often been discussed by writers since the time of Aristotle. Indeed, Laurent in 1894 published a treatise on the subject and an extensive article appeared in the Dictionaire de Sciences Medicale (1812, v. 30). In this country, Hall exhibited to his class in Baltimore a negro of fifty-five who had suckled all his mistress' family (Gould & Pyle, Anomalies and Curiosities of Medicine, Phila., 1897).

When the French Expedition for scientific exploration set out in 1785 under command of Count Jean Francois Golaup de la Perouse, the French Academy of Medicine proposed a long list of questions which the investigators were to look into. Among those propounded by the anatomists and physiologists were the following: "It has been said that men are frequently found in America whose breasts afford milk in sufficient quantity to suckle children; is this true? What are we to think of the hermaphrodites of Louisiana?"

Humboldt, in his personal narratives of 1799–1804 mentioned that in the village of Arenas, near Camana, there lived a man who had suckled a child with his own milk. The mother having fallen sick, the father, to quiet the infant, took it into his bed, and pressed it to his bosom. This man, Lozano by name, then thirty-two years of age, had never before remarked that he had milk, but the irritation of the nipple, sucked by the child, caused the accumulation of that liquid. The milk was thick and very sweet. The father, astonished at the increased size of his breast, suckled his child two or three times a day during five months. Witnesses testified that during this period the child had no other nourishment than the milk of its father. Upon examination 13 or 14 years later, the man's breasts were found to be wrinkled like those of a woman who had given suck. Humboldt mentions accounts of male animals whose breasts contained milk. Among the signs of alleged weakness of Americans, travellers have mentioned the milk contained in the breasts of men. It is improbable that this occurred in any group or tribes. The man in question here was a white man of European ancestry.

In every age, examples are cited of very young girls and of women in extreme old age who have suckled children. Alexander Benedictus, anatomist of Verona who lived about the end of the fifteenth century, relates the history of an inhabitant of Syria, who, to calm the fretfulness of his child after the death of the mother, pressed it to his bosom. The milk soon became so abundant that the father could take on himself the nourishment of his child without assistance. Other examples are related by Santorellus, Faria and Robert, bishop of Cork.

The anatomists of St. Petersburgh have observed that among the lower orders of the people in Russia, milk in the breasts of men is much more frequent than among the more Southern nations. The greater part of these phenomena having been noticed in times very remote, it is not uninteresting to physiology that we can confirm them in our own days.

The Iriquois present an early example of artificial feeding of babies. They made a mixture of butter and hickory nuts which were dried and ground with jerked bear or deer meat. The mixture was boiled and given, when cooked to the proper temperature, in an ingenious nursing bottle. A bit of dried and greased bear gut was used, a goose quill being tied in at one end while the other was tied after pouring the pap (Stone, 1935). The Mead-Johnson collection of Pediatric Antiques displays 9 examples of Indian infant feeding utensils obtained by the dispersal of the Edward W. Payne collection of stone age relics. These were recovered from child burials or are miniature copies of adult utensils leaving little doubt as to their use in feeding children. The first is a crude clay feeding bottle made by the mound-builder Indians of Missouri; the second, also from Missouri mound-builders, is an unglazed pottery feeding bottle with very primitive, somewhat gourd-like containers with the open end drawn out narrow to assist in accommodating itself to the young mother. The third, excavated at New Madrid, Missouri, is a much finer piece of workmanship, also in the shape of a flask with the edge of the mouth scalloped somewhat and quite even. The fourth is a red glazed pottery feeding bowl of the Arkansas mound-builders, somewhat similar to a porridge bowl and from which, perhaps, mushy type of food was given. The fifth is a rare feeding vessel of rust colored pottery from the Oklahoma mound-builder Indians which is also gourd-like in shape but in addition has a hollow spout issuing from the side from which the infant could suck. The sixth is an Indian feeding bottle of painted pottery, slightly glazed, rust and black decoration showing a still further advancement in artistic skill, which was excavated at Newtrozo Creek, Arizona. The seventh, also of the Arizona Indians, although more elaborately decorated

and furnished with a handle like that on a cup, is quite similar in design to the preceding, though painted in brown and white. The eighth object is a papoose spoon very similar to a pap boat, with the handle concave to form a sort of gutter along which the liquid or semi-liquid food could no doubt be guided into the infant's mouth. This is of pottery, decorated in black and white, and came from the Hovenweep Canyon of Colorado. The last is an Indian feeding cup, no handle, its capacity only three tablespoonfuls, made of brown and white painted pottery and coming from Arizona.

In the early days in California nursing bottles were built of tubes of elder with a bladder attached. Nursing bottles were also constructed by the early mothers of this locality from cow-horns, and furnished with nipples made of buckskin. George Darby, writing about the Indians of British Columbia, stated that there were very few cows up the coast and it was very rarely that an Indian child got fresh milk. There were a surprising number of women who could not nurse their children at all, and so condensed milk was used for infant feeding to a large extent, though as soon as a baby was able to cry for it, he was given anything he wanted, even to soda water. There was a high infant mortality, although in the village where there was some supervision they were doing better. The Nanticoke Indians of Delaware made good use of Arrowroot (Peltandra undulata) as a baby food. Yucca-root pap formed a great part of the infant's food in some parts of Central America, even while it was being nursed by the mother. In the region of Valencia, in South America, Humboldt (Travels, v. 2) described a tree the sap of which was a nourishing milk. It was called the cow tree (palo de vaca). The inhabitants of the region drank plentifully of this vegetable milk and considered it a wholesome aliment. When incisions were made in the trunk of this tree, which resembled the broad-leaved star-apple (Chrysophyllum Cainito), it yielded an abundance of a glutinous milk, tolerably thick, devoid of all acidity and of an agreeable and balmy smell. When standing in the air it developed a stringy, cheesy coagulum which grew sour in about five or six days. Nitric acid caused the separation from

the milk of this same viscous membrane. The natives called the tree "Arbol del leche," the milk tree. At sunrise the natives and the negroes of the region collected the milk in bowls and carried this juice home to their children.

Cornplanter, a very intelligent chief of the Senecas said "that one reason why the Indians do not rear so many children as the whites is this: that the children of the former eat quantities of green maize. Certain it is, that the Indians lose great numbers of their children; and I have seen in their villages that the children are often pallid, unhealthy in appearance and very tumid bellied," a clearcut description of nutritional insufficiency by a keen observer of nature (Driscoll, 1933). Among the Cheyennes Mŏksōĭs, meaning "pot belly" was a term of endearment for a little boy, testifying, perhaps, to the commonness of this appearance in the children. Another writer who lived among the Indians of the middle west around 1870 or thereabouts made the following comment: "A somewhat comical characteristic of Indian children is the excessively protuberant abdomen and the thin legs and arms; a fat, chunky boy or girl is a rare sight among these people. As boys are never subjected to bearing burdens, and hardly ever put upon their feet before they are two years old the sight of bowed or knock-kneed legs among them is very rare indeed, but the practice of setting the young girls at the duty, mornings and evenings, of bringing packs of wood and water causes nearly all of them to be sprung either from in or out at the knees" (Wild Babies, Harper's New Mo. Mag., 1878). A pernicious practice, not uncommon to primitive races, consisted in the mother first chewing up the food and then passing it into the infant's mouth. Dr. Charles Blake, writing in 1786, saw syphilis thus transmitted in lower Canada. "The disease (St. Paul's Bay Disease) is nothing more than a confirmed syphilis," he observed; "the habits of the Canadians facilitate its communication in various ways: they use the same cup, drink from the same bucket, chew their infant's food and spit it into their mouths" (Charlton, 1923). Among the Indians in the Honduras, after a child is born, a mixture with supposedly magic properties, although consisting mainly of oil and dirt, is

thrust into the child's mouth (Corlett, 1935). The Lummi Indians of Northwest Washington, on the day of birth, gave the infant to suck some chopped clam meat soaked in the milk of another nursing mother. When an Indian papoose had rounded out its second year of existence, it left its cradle and began to chew meat, fish and other foods. He was seldom seen without some food in his mouth, if food was at all available, and when older, would spend hour after hour roasting over the coals of fire little strips of meat or fish impaled on twigs or forked sticks. When edible fruits and berries were ripe the children gorged themselves near to bursting with these gastronomic delights. Near the Indian camps Grinnell noted that the wild plums seldom ripened because the children picked and ate them green. The irrestible desire the Indians have to flee from society and enter again on a nomad life causes even young children sometimes to leave their parents and wander four or five days in the forests living on fruits, palm cabbage and roots (Humboldt, Travels through South America, 1799–1804). Humboldt saw Indian children of the tribe of the Chaymas at the port of Cumana in the Carribean draw out from the earth and eat millipedes or scolopendras eighteen inches long and seven lines broad (v. 1, p. 157). In times of famine, a not infrequent occurrence in the lives of the Indians, a different picture is painted. Conrad Weiser, to whom Indian life was perhaps more familiar than English, gave a piteous account of their condition in the winter of 1737, when he passed from Tulpenhocking in Pennsylvania on his way to New York. Scattered through the forests, they would fix their camps near a grove of sugar maple trees, the juice of which constituted the only magazine of food upon which they could with any certainty rely. Here the children searched along the lowlands and the banks of streams for nuts and esculent roots, or crowded weeping with their mothers around the traveller, in whose exhausted pouch yet remained a few crumbs of corn meal. A handful of maize steeped in a pot of ash-lye to make a kind of soup constituted to them a most luxurious dish. When the child was older different foods and substances were believed to transfer certain attributes. The heart of a bear was eaten to

attain strength; a fox for craftiness, and so on. Among the Eskimos a father explained that the image of a whale was put in a child's mouth so as to feed him on something that would make him grow up to be a fine hunter. Elisha Kent Kane found Eskimo children of the far arctic north who could not yet talk, voraciously devouring great chunks of fat flesh of the walrus. Among the Blackfeet Indians, as with others, as soon as the child had teeth, it began to get meat in its diet, if such were available at the time. Coras of Mexico prepared a feast in honor of a child when it was one year old and on this occassion for the first time salt was placed in the child's mouth. As soon as the child's teeth were all cut, a similar celebration took place and the child was then given its first meal. The Mendoza Codex appears to show that the diet of an Aztec child was prescribed according to age. In a series of ancient Aztec paintings which give a hieroglyphical history of these people, is represented the manner in which children were brought up, the portion of food allowed them, the labors they were employed in and the punishments resorted to by parents for purposes of correction. At the age of three years, half a cake of bread was allowed at each meal; during the fourth and fifth years the ration was a whole cake and during the sixth and seventh years it was a cake and a half, continuing so until the child reached his thirteenth year. From then on the allowance of bread was increased to two cakes. This bread was usually made from corn or maize and resembled the modern tortilla. Humboldt observed everywhere within the torrid zone of South America in a great number of individuals, children, women and sometimes even full-grown men, an inordinate and irresistible desire of eating earth. A fat clay, unctuous and exhaling a strong smell was the chief type of earth ingested. It was often found necessary to tie the children's hands or to confine them, to prevent their eating earth, when the rain ceased to fall. Some individuals seemed in good health while others would soon fall sick and waste away when they yielded too much to this mania for eating earth. At the mission of San Borja, the writer observed an Indian child of the Guahiba nation who was as thin as a skeleton. He was informed by

the mother that the little girl was reduced to this lamentable state of atrophy as a consequence of a disordered appetite, she having refused during four months to take almost any other food than clay. Twenty-five miles away, the tribe of Ottomacs ate the clay generally, yet seemed to experience no pernicious effects, but presumably these people ate sufficient nourishment of other sorts to preserve their health. They prepared balls of "poya" in which they incorporated maize and other furinaceous seeds thus making up a sort of bread. Children and full grown persons were reputed to have eaten this bread without suffering in their health (v. 2, p. 504).

As we have seen, the Indians were great believers in the hardening processes. Youths were inured to all sorts of hardships so that as men they could be healthy and better warriors. Many tribes were well aware of the fact that a strong, well developed body was better able to resist ordinary ailments than a weak, underdeveloped one. Nootka mothers rolled their children in the snow to make them hardy. From birth the Blackfoot Indian had his body toughened to resist injury, cold and hunger, heat and thirst; rough usage, therefore, became his accustomed mode of living. Later, while yet in childhood, he was schooled to endure pain with stolid indifference. He was plunged into cold water and compelled to swim, while his body was sacrified and stung with various irritating substances. A whimper classed him among the women of the tribe, while the bold, unflinching bearing of the warrior was extolled and held as a model of imitation. Running long distances, swimming against the current or the tide and later performing feats of horsemanship all tended to make the American Indian before and after the arrival of the European, almost invulnerable to exposure, ordinary injury or disease.

Markham describes the formative or hardening method as practiced in the fourth rite of the year by the Peruvian nobility and their kings, in vogue in pre-Columbian days. With great ceremonial fanfare on the part of the priests, the youths were given breeches made of aloe fiber and sewed with sinews of the llama. They then marched to a ravine, where they were met by relatives and friends; here the aspirants were severely

flogged to test their endurance and to initiate them into the hardships of adult life. This was followed by songs and such mirth as the flogged youths could display. More flogging took place upon the return to the village, where further religious ceremonies were carried out. These hardening processes, rather sadistic it is true, with foot races and other enduring feats, lasted for a month. In spite of all this or because it was not continuous, the luxurious Inca nobility were not noted for hardihood; the infant mortality among the common people was unusually high. Both the Mayas and the Aztecs laid much stress on physical training for children.

The Indians of the Amazon region of South America also believed that at the period of adolescence a great physical change took place; therefore, it was considered the most propitious time to cleanse the body from the domination of evil and to start it on its way to motherhood and fatherhood purified by torture, and often the shedding of blood. This consisted of scarification and mutilations of the body, blood-letting, lashing with whips and circumcision. Whether or not circumcision in any of its many forms was practiced much among the Indians is hard to say. Mackenzie said that he could not tell for sure but that the appearance of it was general among those of the Northern Indians of Canada whom he saw (Bancroft, 1882). The natives of Goazacoalco and other places in Mexico practiced a kind of circumcision according to an early account (Bancroft). Las Casas and Mendieta state that the Aztecs and Totonacs practiced it and Brasseru de Bourbourg discovered traces of it among the Mijes. Las Casas affirmed that the child was carried to the temple on the twenty-eighth or twenty-ninth day after birth; there the high priest and his assistant placed it upon a stone and cut off the prepuce at the root; the part amputated they afterwards burned to ashes. Girls of the same age were deflowered by the finger of the priest who ordered the mother to repeat the operation at the sixth year. Zuazo added that these rites were only performed upon the children of great men, and that there was no compulsion in the matter, the parents having the option of having their children deflowered or circumcised at any time within five

years. Clavigero reviewed the subject of circumcision and denied it was ever practiced. Ternaux-Compans, referring to Diaz' statement that all Indians of the Vera Cruz Islands were circumcised, says that he must have confounded the custom of drawing blood from the secret organs with circumcision. Ceremonial drawing of blood in children as well as adults was widely practiced, the blood being drawn from ears, nose, lips and other parts of the body. Cogolludo stated that circumcision was unknown to the Indians of Yucatan. Duran and Brasseur evidently consider the slight incisions made for the purpose of drawing blood from the prepuce or ear in the eleventh month as the act. Carbajol Espinosa, following Clavigero, held the scarification of breasts, stomach and arms to be the circumcision referred to by other authors. In the temple of Huitzilopochtli this last ceremony was carried out on all Aztec children born during the year at a festival during the fifth month of the year. At another festival, the priest of this temple made a slight incision in the ear of the female child, and on the ear and prepuce of the male with a new obsidian knife handed him by the mother; then, throwing the knife at the feet of the idol, he gave a name to the infant after duly considering the horoscope and signs of the time.

Father De Smet in 1856 witnessed a strange ceremony probably of somewhat similar import among the Cheyennes. This was the "ear-cutting of children." This custom appeared to be common among all tribes of the upper Missouri and probable in other places, though there may have been some variety in the form of the ceremony. Among the Cheyennes the mother chose the operator and put the knife into his hands. She extended the child on the skin of some animal, carefully prepared and painted and which the Canadians called "pare fléche." While one of the relations or friends held the infant in a quiet position the operator made two to five incisions in the rim of each ear. These incisions were destined to receive and carry ornaments. The mother made a present of a horse to the operator and another present to each one of the assistants.

Grinnell gives the following account of the same practice. When a little Cheyenne baby reached the age of from three to six months, it was time for its ears to be pierced. A man of importance was selected for the ceremonious ritual and a handsome fee was paid to him. Just as with the Hebrew rite of circumcision, the ceremony was one of joy and celebration. If a man should have his child's ears pierced without this ceremony it would create a scandal in the camp. People would say that he had no affection for the child (Grinnell, 1923). In ancient times the person who actually pierced the child's ears made a long cut in the outer margin of the ear, from the top nearly to the lobe, and the strip on the outside was wound with beads or with brass wire.

When a young girl reached the age of puberty and had her first menstrual period she of course told her mother who in turn informed the father. Such an important family event was not kept secret. It was the custom among well-to-do people for the father to publicly announce from the lodge door what had happened and show his happiness by distributing presents.

Many and varied are the rites which accompany the first menses among primitive peoples. A menstruating woman is almost universally considered unclean and most rituals are in the nature of purifications of one sort or another. The Cheyenne girl unbraided her hair and bathed and afterward older women painted her whole body with red. Then, with a robe about her naked body, she sat near a fire, a coal was drawn from it and put before her and sweet grass, juniper needles and white sage were sprinkled on it. The girl bent forward over the coal and held her robe about it so that the smoke rising from the incense was confined and passed about her and over her whole body. Smoke was similarly used for purification of a woman right after childbirth by many Indian tribes. After this the girl was segregated for four days in a separate lodge. At the end of four days her grandmother, taking a coal from the fire and sprinkling on it sweet grass, juniper needles and white sage, caused the girl, wrapped in a

robe or sheet to stand over the smoke with feet on either side of the coal, purifying herself.

Many were the taboos associated with the menstruating woman and men had to shun her under pain of dire consequences of all sorts. An Indian school-teacher told Elsie Clews Parsons that once one of her Sioux pupils developed a tumor on the neck and his father took him out of school on the theory that the tumor was caused by contact with teachers who observed no monthly taboos (The Old Fashioned Woman, 1913).

Among primitive people, and especially among our Aborigines, the onset of puberty is generally accompanied by more or less ceremonial activity. Accordingly, many varied means are resorted to in different tribes, but all have this in common, that some physical injury must be inflicted on the child in this warfare against the demon. Among the Macus of Guiana, the girl, at the period of adolescence, is lashed with whips after she has been isolated for a week or ten days. This ceremony takes place during the night and the girl is supposed and almost forced to endure this without showing signs of pain. The Piaroas have similar practices. They seclude the girl and make her fast for a few days, after which several old women tie her to a pole and blind her eyes. The women then leave her, for if they are present when the evil demon is chased out of the girl he may enter one of them. A group of men armed with whips and blowing on windpipes complete the ceremony. They begin by striking the girl gently on the thighs, then harder, until the blood begins to flow in streams. Thereupon she is left in the care of a medicine man who stops the flow of blood. A large fire is then made around the pole and the evil spirit is supposed to be burned. Among the natives of Massachusetts, when a maid's menses were first taken notice of, a little house, or rather tent, called a wigwam, was provided for her and she was kept apart by herself. The hair upon the foreheads of the young females was cut in such a fashion, from time to time, that it was impossible to determine the natural appearance (Publ. of the Colonial Soc. of Mass., 1913).

Wallace relates that the Indians girls of the Uaupes also undergo a terrible ordeal. Here the girl is secluded in a dark house with only a small amount of bread and water on which to subsist. After this she is brought out and surrounded by all the relatives and friends of her parents. Each person present lashes her several times across the back and breasts until she falls down either senseless or dead. If she recovers she is given a rest of six hours and the ordeal is repeated. This repetition may be continued for some time. The final act consists of dipping the whips or lashes into pots of prepared meat, after which the novice licks off the liquid. In this way is a woman made marriageable.

A peculiar ceremony is described by d'Orbigny in reference to the Yurucares of Bolivia. Among these Indians, when a girl approaches adolescence, her father shuts her up in a dark cabin built for the purpose. There she fasts for four days. When this time has elapsed, the neighbors are summoned and a quantity of chicha (maized beer) is prepared. The maiden is then seated on a stone, and every guest cuts off a lock of her hair and hides it in a trunk of a tree. When all the guests have so done, they seat themselves around her, whereupon she offers to each a calabash full of chicha. When the gathering begins to become a wassail, each father present performs a peculiar operation on his son, who is seated beside him. This consists in piercing the son's arm through and through with a sharp monkey-bone which has been rubbed with spices. This operation takes place again and again until the young men have their arms riddled with holes. The motive for this operation is to make the young men skillful hunters and the arms and legs of the girls are also perforated in order that they may be brave and strong. For six months after this ceremony girls cover their heads with bark and refrain from speaking to men. Among the Kodiak Island Indians when a girl reached the age of puberty she was confined in a hut built for the purpose, apart from the others and so small the inmate could not straighten her back while on her knees. During the six months following she was allowed a room a little larger but was still permitted no intercourse with anyone. A girl's arrival at the

age of puberty among the Gila nation of New Mexico was a period of much rejoicing. When the symptoms appeared, all her friends were informed of the important fact and preparations were made to celebrate the joyful event. The girl was taken by her parents to the prophet who performed certain rites which were supposed to drive the evil out of her, and then a singing and dancing festival was held.

Another custom is that of scarification. Among the Caribs the hair of the girl is cut off with a sharp fishbone. She is then placed on a flat stone and her flesh scarified with sharp agouti teeth from the top of the shoulders down the back. The incised marks generally consist of oblique crosses. In order that the scars may be permanent ashes are sprinkled over the open wounds. Evidently the girls of this tribe take pride in their scars as much as do the students in a German University.

Similar to these ceremonies, although not so severe, are the puberty trials of the boys. Often the youths are scarified or otherwise mutilated in their initiation ceremonies, and generally they are not admitted among the company of full-grown men until such rites have been performed. Among the Indians of Choco the boys are rigorously dealt with: scarification, tattooing, piercing of the lips for the insertion of ornaments, cutting and piercing the earlobe and flagellation, are required before they are given the title of man. While the boys were quite young, they were previously mutilated by sorcerers of the tribe.

Such were some of the ordeals through which many aboriginal boys and girls passed. The mutilations and accompanying ceremonies were essentially due to the belief in demons and spirits rather than against vicious man or beast. The constitution of the male was not weakened by physiologic processes such as menstruation and childbirth, but men likewise had to acquire through mutilation, the ability to deal successfully with demons in various forms. Even today these customs prevail (Corlett, 1935), as do such practices as piercing of ears, nose and lips, tattooing of face, breasts and various parts of the body, etc.

It is difficult to determine what diseases prevailed among the Indian children. The early Aztec physicians differentiated a considerable number of symptom-complexes as independent affections, with a specific treatment for each one. Among these they distinguished Coriza (especially a variety in the newborn), intestinal worms, discharges from the ear, etc. After a baby was born the mother was placed on a strict hygienic and dietetic regime which included the national drink (corn liquor), while the infant was given even more strict attention, for example, treatment of ocular catarrh (Newburger, 1930). As we have seen, many tribes, in common with primitive races from other ages and different parts of the world, destroyed those born with congenital malformations. Weak and immature babies were quickly weeded out, as a rule, by the hardening processes, but this was not everywhere true, for Heckewelder, who lived with the Indians of Pennsylvania in the early nineteenth century said: "I never heard of any nation or tribe of Indians who destroyed their children when distorted or deformed, whether they were born so or came to be so afterwards. I have on the contrary seen very particular care taken of such children."

Juan Agustin de Morfi, in a History of Texas covering 1673 to 1779, stated that among the Carancaguases were many boys (monoguias) whom the Spaniards of Bejar called hermaphrodites, but it was not known whether they were actually hermaphrodites or not. "These abominable people," he said, "find and conduct themselves in everyway like women. They accompany the warriors on their campaigns, not to fight, but to herd the horses which they steal from us, while they face and take whatever is given them; and principally, to lend their bodies to infamous and nefarious uses. Although the wives abominate this canaille (canalla) they have not learned how to persuade their husbands to do away with them."

Sexual perversion was reported among other Indian tribes in widely separated parts of both continents. A Kodiak Island mother would select her handsomest and most promising boy and dress and rear him as a girl, teaching him only domestic duties, keeping him at woman's work, associating him only with

women and girls, in order to render his effeminacy complete. Arriving at the age of ten or fifteen years, he is married to some wealthy man who regards such a companion as a great acquisition. These male wives are called "achmutschik" or "schopans." They were also known in Unalaska (Aleutian Islands) and among the Tlinkets of Alaska. Among the Central Americas and in Mexico the old chroniclers reported that Sodomy was practiced. The Caciques and some of the headmen kept harems of youths who were dressed as women, did women's work and were exempt from war. They went by the name of Camayoas and were hated and detested by the women.

The Reverend Samuel Lee, writing to Dr. Nehemiah Grew of London in the late seventeenth century, stated that there were no "hermaphrodites, monstrous births but few and none crooked" among the Indians. No "foole" among them but some born deaf, and so dumb: but very ingenious to demonstrate their minds, an obvious reference to the sign language so fully developed among the savages (Publ. of the Colonial Soc. of Mass., 1913). In his New England's Prospect of 1635, Wood wrote: "Yet did I never see one that was borne either in redundance or defect a monster or any that sickness had deformed, or casualty made decrepit, saving one that had a bleared eye and another that had a weune on his cheeke." Morton in a similar blurb entitled "New English Canaan" extolling the virtues of the new land to prospective colonists in 1637 noted that "Not any of them crooked backed or wry legged." In Champlain's voyage occurs the following statement: "Tous ces peuple se sont gens bien proportionné de leur corps, sans aucune difformité." Roger Williams stated that the Indians "have amongst them naturall fooles, either so borne, or accidentally deprived of reason" (Publ. of the Colonial Soc. of Mass., 1913). Tantaquideon, writing of the medical beliefs of the Delaware Indians, stated that congenital malformation rarely occurs, but that such physical abnormalities are attributed to the power of malevolent spirits which afflicted the child because of his parents' failure to fulfill tribal obligations by keeping up certain ceremonies bequeathed to them. Such a physical handicap, however, did not keep an individual from

leading a useful life, for he was otherwise blessed. Delaware mythology reiterates the theme of the weakling child vanquishing his powerful opponents through the weapon of shrewd wits over brute strength (Tantaquidgeon, 1942). Hodge, in his handbook stated that children crippled by accident were treated by parents and companions with the greatest tenderness. That congenital abnormalities did occur among the Indians, then, there can be no doubt.

At the village of Comala in the State of Colima, Mexico, a considerable number of the children were said to be born deaf and dumb, idiots or deformed, and if we may believe the early chroniclers, developed goiters at an early age. No doubt they referred to an endemic area such as is known to occur in other parts of the world. Albinos were described among the Guatusos of Costa Rica by Wafer who related that he saw people "milk white, lighter than the color of any Europeans and much like that of a white horse." Their bodies were covered by a milk white down; hair and eyebrows were white and their eyes were oblong with the corners pointing down. During daylight they were weaksighted, restive and lacking energy, but after sundown their cheerfulness, activity and eyesight returned—the latter being apparently as good as that of other people.

Among the Mayans, a tuft of hair hanging over the face of children often made them cross-eyed. Indeed, mothers are said to have arranged it with a view to this very effect, deemed by them a desirable thing, or to have attached to the forehead a small hanging plaster for the same purpose. The number of "bizeos" treated by Dr. Cabot, who accompanied Mr. Stephens in his excursion through Yucatan, shows that though squinting eyes are still common in the country, the defect has at least lost its charm to Mayan mothers (Bancroft, 1882). In the seventeenth century, the Reverend Samuel Lee of Massachusetts Colony wrote that among the Indians teething was not so difficult and they kept their teeth longer.

Infant mortality everywhere was exceedingly high. Marasmus and diarrheas were commonly mentioned by various observers, as were coughs and other ailments of the respiratory tract. Scurvy among the Indians was frequently noted. Dur-

ing Cartier's second voyage to the region of the Gulf of St. Lawrence in 1535, scurvy attacked the Indians of Stadacona in the month of December, a time when food with vitamin C content was scarce. An excellent description of the disease is given in this report, which, by the middle of the following February, spread havoc among the European voyagers. The children of the Indians are not specifically mentioned but in times of famine, so frequent among the improvident American Nomads, vitamin deficiency diseases most certainly affected young and old alike. The natives, however, were familiar with the antiscorbutic properties of the bark of the white pine by which they cured themselves. The bark and leaves were boiled together and drunk every other day while the dregs were applied to the legs of the affected person. The tree was thought by Cartier to be the Sassafras. It cured the worst of them in six days. Epilepsy struck the Aborigines of America with awe as it did the ancient Europeans who called it the sacred disease. After the advent of the white man, contagious diseases attacked the Indians at times with great violence. Apparently tuberculosis, measles, scarlet fever and smallpox were importations to which the natives had no racial immunity. During the British regime at Kaskaskia, pioneer settlement of Illinois, the Indians complained of the English that "they gave us smallpox, which made all our children die." Kalm stated that the Indians carry their children, when they have the smallpox, into the water and duck them. This was done to cool the heat of the fever (Kalm, 1812).

The first outbreak of smallpox in the West Indies occurred in the year 1507, fifteen years after the discovery of America; whole tribes were exterminated. The complete disappearance of some tribes was due chiefly to smallpox. Cotton Mather, writing of the disease as it occurred among the Indians said that whole families of them all died at one time. "In one of the wigwams a poor infant was found sucking at the breast of the dead mother." In 1635, Le Jeune, one of the Jesuit chroniclers of Canada, said that the sickness began with a violent fever that was followed by a "sort of measles or smallpox." A little Indian girl, who had been baptized and sent to

France, died there of the smallpox. In 1636, the Huron festival season was turned to a season of mourning. The Jesuit missionaries everywhere heard the wail of the sick and dying children; and on or under the platforms at the sides of the houses crouched squalid men and women in all stages of the distemper. The Indians of Canada blamed the disease on the French, saying "that to kill their children, some Frenchmen penetrated the horrible depths of the woods, taking with them the picture of a little child which had been pricked with the points of an awl, and that therein lay the exact cause of their deaths." Probably they believed that the holes bored in the child's picture released the demon of the disease to consume them. The early Jesuit chroniclers go on to state: "In consequence of all these sayings, many had us in abomination; they expelled us from their cabins, and did not allow us to approach their sick, and especially children, not even to lay eyes on them." Deaths among the Canadian Indian children during the early Jesuit days is manifest when it is related: "The number of baptized in this single village, since the last relation, has risen to more than 260, of whom more than 70 children under 7 years died." Father Ragueneau, writing of the state of his mission during this period among a number of French Canadian villages reported in one of his letters: "The cabins of our Christians are the most afflicted with the malady; besides that alone of Joseph Chihouatenhoua, where five children have had the disease. The good Anne sees herself robbed of all her children." Everywhere the missionaries went they found the sick, the dead and the dying. "We meet 2 little children who are dying—prostrate near their mother who is all in tears." "I pass near a cabin where three little children are dying." Father Hierosome Lalemont, in 1640, wrote: "We have baptized more than a thousand . . . most of them went out of this world and among them more than 360 children under seven years . . . without counting more than a hundred other little children, who having been baptized in preceding years, have been harvested by this same disease and gathered by the angels like flowers of paradise." The relation of 1661 states: "In 1663,

among the Iriquois, more than 300 dying children were baptised by some captive Frenchmen" (Heagerty, 1928). The Indians never took kindly to the practice of inoculation for prevention of smallpox when it became rather generally practiced throughout the colonies before the introduction of Jennerian vaccination. They were in great dread of the disease and in many places not only abandoned the patient but even effectively isolated it by building fences and other obstructions around and invoking a strict taboo against the approach of anyone near the person of the victim. Sproat, writing about the Nootka Indians stated that young and old of both sexes were exposed when afflicted with lingering diseases.

Writing in 1870, one observer stated: "Consumption is the great regular scurge of Indian Youth. When left to themselves they know nothing of measles, scarlet fever, whooping cough, but they have mumps, several low intermittent fevers, bad colds and hacking coughs, and worse than all they are very scrofulous and suffer greatly from aggravated eruptions of the skin. But if there are any germs of disease in the air of an Indian encampment such as our children are usually waylaid with in early life, the pappoose is promptly brought down with it and suffers like our little ones, only in the case of measles, unless the weather be very warm and fine, the result is almost always fatal" (Wild Babies, Harper's New Mo. Mag., 1878).

Dr. David Day observed: "It is wrong to suppose Indian children better capable of surviving less careful treatment in infancy than are those of the whites. The former are generally born with less vigorous constitution than the latter; and in taking into consideration the numerous causes of disease and death to which these forest children are subjected, the wonder is, how any survive, not why so many die.

"Among the diseases to which this tribe (the Winnebagoes), are especially subject, and one tending more than all others to produce suffering and death, is scrofula; or, more properly, the strumous diathesis, which may be said to pervade the whole tribe. This disease shows itself among them in all its usual forms; enlargement and suppuration of the cervical and other glands, scrofulous diseases of the joints, tubercular meningitis,

etc., are its most usual forms among children" (The Scalpel, N.Y., 1858).

Eric Stone, too, pointed out that the Indians had no racial immunity, and when yellow fever, smallpox, measles or scarlet fever was introduced by a passing white man the Indian community was decimated by the disease. Digestive disturbances were frequent among the Indians because of the irregularity of the food supply and because of the nomadic existence. Fasts alternated with feasts. Rickets was fairly common, dependent upon their dietary (Stone, 1932).

Among the Indians of British Columbia about 50 per cent of the native babies were born with a bluish discoloration of the skin of the sacral region. It was called the "Mongolian" spots because Japanese and Chinese babies have the same characteristics (Darby, 1933). So far as I know there was no pathologic significance attached to this and I merely mention it in passing. When Frederick Starr, student of Mexican ethnology, was in Yucatan in 1901, he was told that every pure blood Maya Indian had a violet or purple spot on his back in the sacral region. The natives called the spot "uits" (bread), and it was vulgar or insulting to make reference to it. Dr. Baelz, a German physician who spent many years in Japan, long ago called attention to the existence of such spots on Japanese infants, which were so common as to be almost characteristic of that race. In time other investigators reported similar spots on other Asiatic babies and on non-Asiatic babies of Mongolian or Mongoloid peoples. Chinese, Annamese, Koreans, Greenland Eskimos and some Malays are known to have such spots. Sacral spots are also reported to have been reported among Samoans and Hawaiians. Because of this dissemination and because they are limited to the Mongolians, the occurrence of the spots has been considered by some to be proof of Mongolian origin. Baelz found two cases among Vancouver Island Indians. Starr, who affirmed that this phenomenon did not occur at all among the Aztecs of Mexico, stated that among the Mayas, the case is different. "In the little Maya town of Palenque, I examined all the pure blood babies. The back of the first little creature bared for my inspection bore a clearly defined, dark

blue-purple spot, just where it might be expected. The spot was almost two inches wide and nearly three-fourths of an inch high. The child was a boy of eight months. A brother, two years old, showed no trace of the spot, but the mother says it was formerly well defined.

"Everyone of the seven pure Maya babies, below ten months old, in the town were purple spotted. A pair of boy twins, two months old, were marked in precisely the same place with pale blue-purple spots of the same size and form. In one boy of ten months the spot seemed to be disappearing and was represented by three ill-defined and separated blotches." Three babies of mixed blood showed no trace of the colored spot (The Chicago Tribune, January 11, 1903).

Of the Tlinket babies, farther to the North, Marchand drew a sad picture. He said the little bodies were so excoriated by fermented filth, and so scarred by their cradles that they carried marks to the grave (Bancroft, 1882). From lists of remedies we learn that Indian children frequently suffered from worms, toothaches, earaches and numerous intestinal ailments common to all infants. The broad teeth of Moose fawns were hung about the necks of children when teething. Heckewelder reported of the Indians of the Middle Atlantic states at the end of the eighteenth century that, "worms are a very common disorder among Indian children, and great number of them die from that cause. They eat a great deal of green corn when in the milk, with beans, squashes, melons and the like; their bellies become remarkably large, and it is probably in that manner that the worms are generated. I rather think that Indian children suffer less in teething than the whites." Throughout Central America many travellers reported that children suffered a great deal from worms and that their abdomens were sometimes enormously swollen.

The first tribes to encounter the white man in the Carribean Sea defended themselves against the incursions of the newcomers fairly well until the introduction of measles among them. Then they succumbed by the thousands. This new weapon proved to be more deadly when coming in contact with a new race than did the cannon and sword of the Spanish

Conquistador. Then came smallpox and whole islands and sections of country were depopulated. In like manner scarlet fever, tuberculosis, and to a less extent, syphilis helped complete the process (Corlett, 1935). When smallpox appeared in Mexico for the first time in 1518, the Indians named the disease the Great Leprosy, in contra-distinction to the Small Leprosy, a term applied by them to measles, of which an epidemic appeared soon afterward (Flores, 1891). Measles was reported in San Domingo in 1519, in Guatemala in 1523 and 1530 when a decree ordered patients to be properly cared for; and in 1558, 1576 and 1826; in Mexico in 1531 and 1595; in Argentina, 1634; Peru, 1558, 1585, 1618, 1693 and 1796; in Panama, 1606; Ecuador, 1611 to 1612, 1692 to 1694, 1785 (Moll, 1944). In Central America the ruling diseases among the native races were said to be smallpox, which wrought yearly havoc; dysentery, which also was not uncommon in the highlands during the summer, and leprosy, attributed to filth, immoral habits and bad food. Father De Smet, traveling among the Indians of the Middle West about the year 1838 wrote: "It is sorrowful to see the neglected condition of the little children in all the Indian Villages. Their hair seems never to have undergone the operation of the brush, so that their heads look like masses of cobwebs. Many have eye trouble and their faces and all their limbs look as if water had never touched them. The younger ones are generally naked." Travelling in the Middle West about 1865, Father De Smet visited various tribes. Upon his visit to the Sioux over 200 children were brought to him to be baptised. "Having scarlet fever in their camp, I spoke to them of the necessity and urgency of baptism and of the eternal happiness of the children who might fall victim to the disease." At Forts Berthold and Sully, sickness had carried off a majority of the children of tender years. At Fort Union, too, the mortality among the Assiniboin children had been equally great. At Masaya the death rate among children was said to be excessive and Scherzer, in his Wanderungen stated that all of the family had an extremely unhealthy appearance, particularly the children who were pallid and lean, had thick, swollen abdomens which he thought was caused

by eating qucca roots (Bancroft, 1882). In the St. Paul's Bay region of Canada, a disease, undoubtedly syphilis, was widespread from about 1755 to 1783, especially among the Indians. Children formed a large proportion of the infected. Some children seemed to be cured of the disease by the medicines which their infected mothers had taken, although the mothers themselves were not always perfectly cured by them. Dr. Bowman observed that none was found to be cured radically without mercury. Children were cured by sucking the mother to whom mercury was administered (Heagerty, 1928). Syphilis was very common among the Indians whose standard of morals condoned promiscuity to such a large extent. Among the Navajos, Mojaves, Yumas and Comanches, prostitution was rife; whole bands were said to be affected with syphilis and its effects often were visible in their young.

In 1722 the Reverend John Ettwin, leading a group of Moravian Indian converts, wrote that the beginning of the journey was not auspicious, as the measles broke out. "The epidemic proved a hindrance to our progress, as it attacked both adults and children. The former had to lay by at least two or three days, after being taken ill, and the children had to be carried. Matters, however, went so well with the sick as to be cause of surprise; all that they required was a drink made of the large sarsaparilla which proved efficacious in bringing out the eruption."

The early Indians of California also suffered severely of the measles (Harris, 1932). Father Ramon Abella, on December 31, 1806, reported 236 deaths from this disease among the Indians there in nine months, of which number 73 were children. Dr. Rollin in 1786 found hernias among the children of the Monterey Indians, though whether these were inguinal or umbilical is not stated. He said that hernias in children were ignored; he had attempted, he feared unsuccessfully, to teach the parents how to reduce hernias in children by taxis and bandages (Harris, 1932). In the early nineteenth century the Rev. John Heckewelder, in commenting on the frequency of crippling rheumatism among the aged Indians, also stated, "I have seen boys 10 and 12 years of age, who through colds or fits of

sickness had become so contracted that they never afterwards recovered the use of their limbs." Paralysis was believed by the Delaware Indians to be the result of an encounter with an apparition. Zeisberger stated that it was claimed that wandering spirits and ghosts sometimes threw something into a public path and whoever passed over it was bewitched and became lame or ill. Among the Delawares the children were cautioned against answering the call of an unseen person as it might prove to be a "ghost-whoop." Should a child answer such a call, his jaw would become dislocated or even his entire body paralyzed (Tantaquidgeon, 1942). When two Dutch travellers visited the site of Albany, New York in 1680, a certain half-breed Indian woman came with her little boy about four years old, who was dumb or whose tongue had grown fast, to ask them for advice or a cure, but as they were not doctors or surgeons, all they could do was to give her their opinion and what they thought of the case (Dankers, 1867). The Aztecs of Mexico believed stammering was caused by too long suckling. Considering the length of time that often elapsed before their babies were weaned, this trait should have been very common.

To understand the theory and method of Indian therapeutics it is necessary to know the mind as well as the means used by the natives in treating diseases. As Stone says, the theological-physical dualism of the American Indian is best illustrated by his use of the word "medicine." He may use the term to refer to an herb or drug, but more often it means some supernatural agency or article which may be of aid in curing disease; just as often the same thing may be invoked to insure the success of some individual or tribal undertaking. The Indian boy of almost every tribe, before achieving manhood, was expected to retire to some secluded spot and "make his medicine." There he fasted, prayed, mortified his flesh, and meditated until, in the auto-suggestive state of ecstasy thus aroused, he received some communication from the gods; the appearance of some animal, a bird, some unusual conjunction of the elements, or some fantasy which would reveal to him the supernatural agent guarding and directing his life. This would become his "medicine," and would in future be invoked in times of social, mental

or physical stress. Among the Pawnees when a child fell ill, medicine men were called in and the supernatural agent called forth by the medicine man who effected the cure was understood to be the fetish presiding over the child's destinies (Stone, 1932). When an Indian sorcerer was examined in 1690 by Francisco Antonio Fuentes of Guzman as to the manner of assigning the proper nagual to a child, he gave the following account:

Having been informed of its day of birth, he in due time called at the residence of the parents and told the mother to bring the child into the field behind the house. Having there invoked the demon, the NAGUAL of the child would appear under the form of the animal or object set opposite its birthday in the calendar, a serpent were it born on the second of January, a flower were it on the thirteenth, fire were it on the twenty-fourth, and so on. The sorcerer then addressed certain prayers to the NAGUAL to protect the little one, and told the mother to take it daily to the same spot, where its NAGUAL would appear to it, and would finally accompany it through all its life (Brinton, 1894).

When a child was sick and the mother's or friend's ministrations proved ineffectual, the shaman or medicine man (who could, in many tribes be a woman) was called upon for aid. Many women of the Ojibway tribe took the degree of Midiwin in the "Grand Medicine Lodge" and of course a large part of their practice was restricted to women and children.

The following description written in 1870 was evidently by an eye witness: "When a papoose becomes ill, refuses to eat or be comforted, and after several days and nights of anxious, tender endeavor to relieve her child, the mother begins to fear the worst, she sends for the shaman, or a doctress of the tribe, and surrenders her babe to his or her merciless hands. This shaman at once sets up over the wretched youngster a steady howling, and then anon a whispering conjuration, shaking a hideous rattle or burning wisps of grass around the cradle. This is kept up night and day until the baby rallies or dies, one

doctor relieving the other until the end is attained, and the result is death nine times out of ten.

"The Indian sees in a sick person the form of one who is stricken down by the lodgment therein of a devil or bad spirit and the only way to restore the patient to health is to scare this devil, terrify this demon, out of the body of the sick, and to do this their infernal practices are resorted to dancing around the sick and dragging them about, yelling and frothing at the mouth and making hideous noises with calabashes and rattles day and night, without a moment's intermission.

"If the patient then recovers, the shamans are warmly congratulated; when the sick die, however, under this peculiar treatment, there is no reproach uttered, no hint as to the least desire to change doctors when the next case comes along, but on the contrary, the shamans are gravely and humbly complimented for their wonderful courage in attacking and facing so powerful and wicked an evil genius as the one must have been which succeeded in taking the life of the patient in spite of the doctor's terrible adjurations and noisy incantations" (Wild Babies, Harper's New Mo. Mag., 1878).

Eric Stone presented a similar picture somewhat more vividly: "In 1887 a band of Chiricahua Apaches were confined as prisoners at Fort Marion (St. Augustine, Fla.). An epidemic broke out invaliding about half the band and causing the death of 23 children. At last the medicine-man decided to hold a 'cha-ja-la.' The preliminaries were almost as important as the ceremony itself and both were directed by Ramon, the oldest medicine-man, who drummed and chanted from the first application of paint till the last ministrations were completed. The ritual began with the adornment of the three shamans who were to be the actors in dance. They all wore undercoats of greenish brown. On their bare upper arms was painted a yellow snake, its head over the deltoid, and a red flannel ribbon was bound on below this. On the breast and back of each jacket were painted various insignias about four inches in diameter. On the breast of one was a yellow bear, and on his back a Khan; bear and zig-zag lightning adorned the second,

while the third was decorated, front and back, with the conventional lightning design. Each wore a different mask and each bore a wand in either hand, about the proportions of a common lathe, with a snake lightning design on both flat surfaces. When ready, the medicine-men began a peculiar whistling as they bent slowly left and right; then backward and forward until the head was at a level with the waist. In this position they spun rapidly in a circle on the left foot and then in reverse direction and charged around, in and about the group of tents occupied by the band, making cuts and thrusts with their wands to drive out the evil spirits. Then they joined and visited a squaw who held a papoose on a cradle. The mother remained kneeling as they frantically beat about the baby with their wands. Then the squaw held the cradle up to the four cardinal points of the compass and at each point the play with the wands was repeated. At each point, after passing of the wands the baby was given to each medicine-man who held it to his breast, lifted it to the sky, lowered it toward the earth and then held it out to each of the cardinal points. All the time and during all this maneuver the medicine-men were whistling, snorting and prancing to the rhythm of Ramon's drum. The mother and friends of the patient's family added to the noise by shrieks and cries. Each invalid was visited and the same procedure was carried out over each."

Other measures were used in various ways as in the following by Musters (quoted from Corlett, 1935).

"The patient, a child about a year and a half old, was very ill with influenza. The doctor arrived, and laying the child on its back, proceeded, after patting it lightly on the head, and murmuring an incantation, to place his mouth close to the patient's chest, and shout, as far as I could understand, to exhort the evil spirit to leave the child; after this he took it up, carefully handed it to its mother who, under his directions smeared it all over with gypsum. This over, it was handed back to the doctor, who had been absent a minute. He then produced a hide-bag, at the bottom of which were some charms; into this he inserted the baby's head several times, muttering incantations; after this a white mare was brought up, and after being

painted with red ochre hand-marks all over, was knocked on the head, cooked and eaten. The liver, heart, and lungs were hung on a lance, at the top of which was suspended the bag containing the charms. Whatever effect these ceremonies may have had, the child recovered."

The Indians were firm believers in the curative value of the "laying on of hands" especially by those endowed with special virtues. When Cabeza de Vaca was stranded for six years among the Aborigines of Texas, beginning about 1529, he was put to work by the savages curing their ills by placing his hands on various parts of their anatomy. Calling upon his God and Saints may have been his own idea, but his methods were curiously akin to those of the Indian medicine men. When Jacques Cartier, French voyager, arrived in Canada in 1535, there came out of one village all the women and maidens, "part of which had their armes full of young children, and as many as could came to rubbe our faces, our armes, and what part of the bodie soever they could touch, weeping for very joy . . . desiring us with their signes, that it would please us to touch their children." The significance in this is later brought out when "they (the Indians) brought before him (Cartier) divers diseased men, some blind, some criple, some lame and impotent and layd them all along before our Captaine, to the end they might of him be touched: for it seemed unto them that God was descended and come downe from heaven to heale them. Our Captaine seeing the misery and devotion of this poore people, recited the Gospel of St. John, that is to say, In the beginning was the word; touching everyone that were diseased . . ." Of the Indians of California, Sir Francis Drake reported in 1579 "they then began to shew and make knowne unto us their griefs and diseases which they carried about them . . . making signes, that if we did but blowe upon their griefes, or but touched the diseased places, they would be whole." Drake, however, while fully aware of the value of the "King's Touch," resorted to the commoner healing practices of Europe of his time, for he used "ordinary meanes, as lotions, emplaisters and

unguents, most fitly (as farre as our skills could guesse) agreeing to the natures of their griefes, beseeching God, if it made for his Glory, to give cure to their diseases by these meanes."

In many parts of the continent mud baths, medicated baths, sweating lodges, and other forms of vapor baths were used extensively, steam being generated, as a rule by pouring water over hot stones. This form of treatment was used for many ills, but the white men living among the Indians thought that measles and other eruptive fevers treated in this way almost always proved fatal (Andrews, 1903). They were undoubtedly prejudiced in the general belief that this would induce more "cold." Measles is met with so frequently as the diagnosis among the Indians anyhow, that one is led to believe it may have been hodge-podge of many eruptive fevers. In cases of measles some Indians used to fix a half-dried castor of the beaver on the end of a needle and stick it up each nostril to relieve the congestion and inflammation. One motive of the Acyuses in the massacre of the Whitman family is supposed to have been the missionary's failure to cure the measles in the tribe. He had done his best to relieve the sick and his power to effect in all cases a complete cure was unquestioned by the natives. Among some early Indian natives ailing infants were washed in urine, and according to Garcilaso, even made to swallow some of their own discharges (Moll, 1944). Some early natives of Latin America, when children were taken sick, had their tongues examined and if they were coated, the children were given a piece of dried cord to suck. Aleutian Island mothers plunged their crying babies naked into the water, winter and summer, and held it there until it was quiet. No doubt about the effectiveness of such hydrotherapy (Bancroft, 1882).

When the United States Government began to erect hospitals in the Indian reservations it was indeed an innovation. The establishment of a white man's school for their children and a new medicine house aroused a great commotion among the Indians of one reservation. When a man died in the hospital of acute appendicitis, the Indians refused to go into the building.

"What," said they, "do they want us to go in a dead house when we are sick. NO!" However, this abhorrence of a hospital or building in which someone has died is by no means peculiar to the Indian races. Time and time again I have met with strong opposition from my own patients against sending a child to this or that hospital because the parents or neighbors knew of someone who had died there.

When an Indian boy was taken ill suddenly while at school the weather being unfavorable and his tepee some miles away, he was induced to go into the new hospital for the night. He grew rapidly worse, his temperature shot up to 104 degrees and towards morning he became delirious. He called for his mother and begged to be taken home. The doctor pronounced it pneumonia. The outlook for both the patient and the hospital looked bad. Should an Indian die in the new hospital, its use as a hospital would have to be abandoned.

In the early morning the mother came. Mokuyi-Kinasi, the medicine man came too. He asked that the boy be allowed to be carried home. "For many generations of men the Great Spirit has caused the corn to sprout and bring forth a plenteous yield. He has also given us herbs to cure the sick. We do this in our own way. We do not believe in keeping house for our people to die in. The bottles smell and let out 'bad medicine.' We do not like it, let us go." The nurse remonstrated; with her shrill, strident voice; she said, "It will kill him to be moved." The doctor, evidently not hopeful as to the outcome of the boy's illness said, "The old cuss"—referring to the Indian doctor—"might do him good." And so it was arranged that the boy should go. He was tenderly wrapped in blankets by his father, mother and grandfather, and carried to his home. Once there, the medicine man cupped the chest by powerfully sucking different parts, gave him a drought and softly crooned a medicine-song accompanied by the gentle rattle of the medicine-che-che-quay. The boy swooned into a sleep, from which he awakened somewhat refreshed. He was contented for he was at home among familiar objects. The odor of the bottles in the apothecaries' room was replaced by sweet smelling grasses. His ear caught the mocking bird's morning song.

There was no foreboding, sour-faced nurse, but his mother bent over him and with loving hands smoothed his brow. The kind, wrinkled face of his grandfather looked down on him on his cot of furs. His heart gave a joyous throb; he felt better. A sweet langour replaced the fever which bore him farther and farther away from the white man's school and the white man's dead house. He made a rapid recovery under Mokuyi-Kinasi's care.

So far as I could ascertain, Mokuyi-Kinasi gave nothing more than curative surroundings, the personal influence of those in whom confidence was reposed, wrought what "the dead house" and the nurse, whose personality was "bad medicine" even to the less acute Caucasian, could not supply and were deadly when brought in contact with the intuitive Indian who read at a glance impending doom (Corlett, 1935). This calls to mind too, Dr. L. Emmett Holt's frequent prescription: One hour of loving care.

The Hopi Indians were particularly fond of practicing according to the "doctrine of signatures." For convulsions a twisted piece of wood was laid on the body; in some Indian tribes, mothers with faulty lactation were given concoctions of milk weed. In any form of gastric disorder the Indians employed emetics. In fact the Indian took emetics as frequently and as casually as the white takes laxatives. The Apache hung a small stick from the woody part of a spiny cactus called cholla (opuntia emorcyi) around the necks of their children to protect them against sickness.

Among the Mohaves when a child became afflicted with whooping cough the father must perforce abstain from drinking tea, and had to bathe in the Colorado River at specified intervals. A vaguely similar custom was found among the Winebago. The Delawares thought relief could be obtained in whooping cough if the mother first suckles a puppy and then her child. Chestnut (Castanea dentata) leaves made into a tea were used by the Mohegans to cure this disease, while the Delawares considered a tea made from the root of the Skunk Cabbage (Symblocarpus foetidus) to be the remedy of choice. A number of methods are in use among the Nantikoke Indians

to avert or cure whooping cough. It is believed that a string or necklace of deerskin will protect the wearer against this disease. If a woman marries without changing her name, i.e., marries a man of same name, she can cure her children of whooping cough by feeding them bread stolen from her neighbors. One informant stated, however, that it is bread stolen from such a woman that effects the cure. In the first instance, a friend often was asked to leave bread where it could be taken unseen.

Another method of treating whooping cough involved securing as many cockroaches as there are children affected, and naming one after each child. Then each child placed a roach into a bottle which was then tightly corked. The sickness is believed to pass with the death of the insect. During this period it is necessary to keep the child's bowels open, else the charm may react and kill him.

A person living in the city is advised to put a cockroach in a thimble, tie it up in a cloth and wear it around the neck, "you will never whoop after wearing it." Whooping cough has been prevalent probably since the discovery of America. It was mentioned in Haiti in 1770, in Brazil in 1780 and in Costa Rica in 1801.

For chickenpox, the child affected is placed before the open door of a chicken house so that the chickens in coming out will fly over him.

Blood-letting by scarification, suction and other means were not unknown to the Indians of North and South America. However, an irate aboriginal father nearly strangled a French Priest to death when one of his children received this treatment for an illness which subsequently caused its demise.

In an anonymous Hispano-Mexican manuscript discovered at the Biblioteca Nazionale Centrale in Florence, the text relates: "When a child has a sore chest, the sorcerer uses the herb iztauhiati, and sucks out blood or matter. Some children get well. Others do not" (Nuttal, 1902). Bernardino de Sahagun, a Spanish Franciscan friar of remarkable intellectual gifts, who went to Mexico soon after the conquest and left a valuable work on the customs and history of Ancient Mexico,

recounted the following curious mode of treatment for children: "The child patient, or the patient child, was held head downwards and its head was shaken to and fro, its mouth being pressed close or stopped with cottonwool. Of this prescription it may be surmised that some may have recovered, but that the majority did not." The Incas of Peru attributed some healing powers to the umbilical cord and allowed the infant to suck upon it if it became ill (Ploss, 1876).

From the extensive materia medica of the native Indians published in different places a few substances stand out as having been particularly useful in children. Sour Dock (Rumex crispus-1) was widely used for diarrheas and other diseases by the Indians and the early pioneer American Settlers. A decoction of the root of the Wild Licorice (Glycyrrhiza Pepidota Pursh) was used as a remedy for fever in children; Sarsaparilla was also very popular. The root, bark and inner bark of the trunk of several species of oaks were used by many tribes in the form of a decoction for diarrhea. The root of the Raspberry (Rubus occidentalie L. & R. Strigosus Michz.) was similarly prepared as a remedy for bowel trouble and an infusion of the leaves combined with willow bark was considered an excellent remedy for cholera infantum by the Crees. The bark of mountain ash (Sorbus Americana, "bear berry") was steeped in hot water by the Indians in the region of St. John's Lake, Canada, and given to babies to drink for general disorders and especially for cholera. Red alder bears the same name and served a similar purpose. Cogswellia orientalis (C. & R. Jones) as a tea was used chiefly for children to relieve pain in the bowels or diarrhea. The plains Indians called the Parnasuie fimbriata (Konig.) "for children to drink" (kas kun isesti man i wats). From the powdered leaves of this plant a tea was made which was administered to very small babies when dull or when sick at the stomach (Grinnell, 1923). A strong decoction of the chocolate root (geum nivale) was used by the Indians of Newfoundland for dysentery, colds and coughs, particularly in children. The Arabis glabra (L) was used to check a cold when it first appeared and as a general preventive of

sickness; by the Cheyennes it was "given to children when sickness was about." The Seneca tribe employed the root of the Seneca Snakeroot (Polygala Senega L.) in the form of a decoction as a remedy for coughs and colds and the leaves in the treatment of sore throats. Skunk-oil was regarded by the Delawares as a valuable remedy for colds and croup. For children the average dose is from 3 to 5 drops. A small quantity may be used externally on throat and chest. For colds, a syrup made by roasting onions was also used. For infants and young children the syrup was sweetened and a few drops taken clear or in milk. A tea was made also from Calamus or "muskrat" root (Acorus Calamus) to cure infants of colds. A tea brewed from "Horsemint" (Monarda punctata) or skunk cabbage leaves was also good for colds (Tantaquidgeon, 1942). The Indians now living in Oklahoma give babies the grated root of the arrow-arum (Peltrandra irrginica) with milk. If a child suffers from croup, he is stood against a door-joint and his height marked. After he outgrows the mark, the croup will leave him. The Aztecs believed morning dew cured catarrh in newly born children (Bancroft, 1882). The Prairie mushrooms or puffballs (Lycoperdon Gemmatum Batch.) were gathered and kept for use as a styptic for wounds, especially for application to the umbilicus of new-born infants. The powder of these fungi was used as a dusting powder to prevent chafing of the skin. For earache a wad of yarrow leaves (Achillea millefolium L.) was crushed and put into the ear (Youngken, 1876). An Indian treatment for earache that Dr. Corlett remembered to have undergone as a small boy was as follows: A green limb was cut from an Ash tree and one end put into the fire. As the sap was driven to the free end by the heat it was allowed to fall drop by drop into the upturned ear, the patient lying on his side (Corlett, 1935). The Delawares used chestnuts (Aesculus glabra), "bigacorn tree," ground up and mixed with sweet oil or mutton tallow to make a poultice for earache. A small bag filled with hops (Humulus lupulus) and heated was also considered useful for earache or toothache. Skunk scent or rabbit urine was put into the ear to relieve earache by some modern

tribes. The Maricoba tribe prepared earache drops from the seeds of the castor oil bean while the Montagnais put skunk oil into the ear to relieve earache. Others blew tobacco smoke into the ear for the same purpose, a practice I have seen used among my own patients. A little bag of dried blossoms of Hops (Humulus lapulus) was applied, heated, in cases of toothache or earache by the Mohicans. The Omaha and Ponca tribes powdered blue flag, mixed it with water or saliva and dropped this preparation in the ear for earache.

Various tribes of North American Indians rubbed the soft down of the cat-tail plant on the skin of infants to prevent chafing. Newly born babes were laid in a mass of the down. Paint was put on the faces of both adults and children as a protection against wind and sun as well as for many other reasons and among the Apaches and Mojaves it was a common practice to plaster the head and body with mud as a protection against vermin and the sun's rays. Cochimi and Pericui mothers of New Mexico, for protection against the weather, covered the entire bodies of their children with a varnish of coal and urine. The primitives everywhere lived in filth and squallor and vermin abounded. The people of Tehuantepec and of Chiapas were even pictured as having a disgusting habit of eating the vermin which they picked from the heads of their children.

The Montagnais of the region of Quebec boiled the leaves of the pitcher plant (Sarracenia purpurea) and used the tea as a wash for sores and for children's rashes. For the same ailment a leaf of the plant could be split and bound over the affected part. It was said to draw out the "bad stuff." Ringworm was cured by California Indians by placing the milk of the poison oak in a circle around the affected part. For infantile skin eruptions the ancient Mayans advised anointing the body with a poultice made from the seeds of the michi-husuhtli, red incense, grain which were to be burned; then the plant tlatlanquaye, leaves of the huitzitzilxochitl, the root of the tlalahuehaetl and tla-yapaloni, laurel leaves, xiuh-ecapatli leaves from which the acid water is to be drawn; the boy was also to

drink medicine from white earth, the small white stones gathered from the bottom in flowing water, the stone a-camallo-tetl and coltotzin, the bush tlalmizquitl, and points ground in water. When the infant refused to breast because of pain or when the infant was so affected that it spewed out the milk and would not take the breast into his mouth, he was given a drink made of the herb called te-amoxtli, quail's blood set in the sun, and its hairs, somewhat restored, which were to be incinerated. Also a poultice was ordered to be carefully prepared from a weasel's brain and a scorched human bone, from which the acid water was to be drawn (Gates, 1933).

The Cheyenne tribe believed the rhizome of the Sweet flag (ocorus calamus or Calamus), when chewed and rubbed on the skin, to be good for any malady. They tied portions of the rhizome to the dress, blanket or necklet of their children to keep away the night spirits (Youngken, 1876). The Delawares prepare the leaves of catnip (nepeta cataria) with peach seed to make a syrup very beneficial to children.

Among the Cherokee Indians (Mooney, 1932) the very strong sinewy roots of goatsrue of catgut (Tephrosia, Virginiana L.) are boiled and given to the child to drink to make it strong and muscular; it is given the eavesdrop, from where it falls in one continuous spout, to drink to make it a fluent speaker, although this belief was probably borrowed from the whites. The fleshy tubers of the wild lily (Lilium canadense L.) are boiled and the decoction is given to the child to drink; he is also bathed in it, the object of both actions being to make it fleshy and fat, by the same reasoning that eating the heart of a bear will endow strength, of a fox cunning, etc. Another plant put to the same use as the wild yellow lily bulbs was the Adam and Eve or putty roon (Aplectrum hiemale). The fronds of brake (Pteris sp.), from the stiffness of the leafstalks, came to be thought of for weak babies and for old people; they were made up into a bed for the patient. Some tribes in South America smeared children, frail individuals and sick people, with the castigation blood in order to make them courageous, strong and healthy. The Montagnais Indians of Canada drank the sap of the poplar (populous tremuloides) for worms. The bark

also was steeped for the same purpose and used as a drink. Wormroot (Apocynum cannabium) also was used, and snakeroot (aristolchia serpentaria) was given for fits because of its twisted appearance. Rhubarb was highly lauded by Monardes who wrote: "The whiche beying used of many, the fame of it was extended all abrode, that in shorte tyme, banishying the use of Ruibarbe of Barbarie, and tamyng it Ruibarbo of the Indias, and so all men dooeth commonly call it. And also it is called Mechoacan, for that it is brought from thence; and not only in Mexico, and in that countrei thei doe take it as the most excellent purgation: but also in Peru and in all the partes of the Indias.

"It is given at all Tymes and in all ages, it dothe his woorke without molestation. It is a medicine easie to bee taken, for that it hath no evill taste. . . . and so it is easie for children" (Monardes, 1925). George Sticker, in a paper on the diseases in Central America at the time of Columbus published in Janus (1924, v. 28, p. 232) quotes from Piso, "De Indiae utriusque re naturali et medica, (Amsterdam, 1658) the following in regard to ipecocuanhoe: ". . . this salutory root, which has not only the faculty to expel all noxcious matter per superiora et inferiora, but also resists all sorts of poisons. I believe that a more excellent medicine against diseases arisen from old obstructions, or to cure diarrhoeas scarcely may be found . . . as it upsets the body less but opposes effectually all poisons, it is principally administered to children and grand women." This root had such a salutary effect that the Brazilian Indians, from whom Piso learned its use, kept it as a holy secret (Patten, 1930). Now it is a popular household remedy present in many a medicine cabinet of homes where there are young children present. Giving an early account of an Indian remedy, Peter Kalm, from his travels in America in 1748 reported that: "The Worm-seed is plentiful on the road, in a sandy ground such as that near the ferry, opposite to Philadelphia (on the Jersey Shore). I have already mentioned that it is given to children to carry off the worms. It is then put into brandy, and when it has been in it for one hour, it is taken out again and dried, and given to the children, either in beer, sweetened with treacle,

or in any other liquor. Its effects are talked of differently. Some say it kills the worms; others again pretend that it forwards their increase. But I know, by my own experience, that this worm-seed has had very good effects upon children."

Peach leaves (Prunus persica) are infused by the Delawares to expel pin worms and to stop vomiting in children. A small quantity is sweetened and drunk occasionally until relief is obtained (Tantaquidgeon, 1942). Button Snakeroot (Eryngium aquaticum) was also used for pin worms as well as for tapeworms. Tobacco was also used "In wormes, and in all kinde of theim, it killeth them and dooeth expell theim. Merveilouslie, the seethyng of the hearbe made a sirupe delicately, taken in verie little quantitie, and the joyce thereof put on the navell, it is needfull after this be doen to geve a glister, that may avoyde them out, and expell them out of the guttes" (Monardes, 1925). Some other plants used by the Aborigines for worms, which must have been quite common among them, were: running blackberry (Rubus hispidus), Spearmint and peppermint teas, "peppergrass" (Bursa bura pastoris), leaves of spicewood which were to be brewed or made into a tea, and others.

Spigelia was valued as a vermifuge before the discovery of America and was also used as a sedative and diaphoretic. A decoction of the rhizomes and roots of the smaller yellow lady's slipper in which was placed red fleshy stalks of a chickweed was used for worms in children. From the appearance of the red, fleshy stalks of the chickweed, it was supposed to have some connection with worms (Youngken, 1876). In 1764, Alexander Garden, M.D., of Charlestown, South Carolina, published an enthusiastic account of the Indian Pink in which he said: "About 40 years ago, the anthelminthic virtues of the root of this plant were discovered by the Indians; since which time it has been much used here by physicians, practitioners and planters" (Essays and Observations, Philosophical Soc. of Edinbourgh, 1771).

Tobacco was used for many ills by the Indians long before the coming of the white men. Monardes wrote: "In one thing, the women that dwelleth in the Indias, doeth celebrate this

hearbe, that is in the evil breathyng at the mouth of children, when thei are over filled with meate, and also of greate people, anointyng their beallies with lampe oile, and puttying some of those leaves in ashes hotte to their beallies, and also to their shoulders it doeth take away their naughtie breathyng; and it doeth make that thei goe to the stoole, the tymes that it is needfull" (Monardes, 1925). The same writer mentions an oil from "The Figge Tree of Hell" applied to the navel of children "doeth provoke them to the stoole and if they have wormes it doeth expell them and killeth them" chiefly if given a drop or two in milk. The present day Nanticoke Indians of Oklahoma still blow tobacco smoke into a child's mouth to cure it of colic. For indigestion of babies the following "tea" is considered beneficial: A crust ("crisp") of soot taken from the chimney is placed in a cloth bag and steeped in a half cup of hot water. The Cherokees used a decoction of Cranesbill along with chicken grape as a mouth wash in thrush and Canada Moonseed was used for scrofula and sore mouth. The Mohegans made the leaves of the rattlesnake plantain (epipactic pubescenes) into a mash to prevent sore mouths in babies and gave "Elder Blow," flower of elder (Sambucus canadensis) made into a tea to babies with colic. As a mouth wash for infants they used an infusion of Golden thread (coptis trifolia). For colic they also gave their babies catnip tea (Nepeta cataria), while the Teton-Dakota tribe administered a decoction of grindelia to their children for this complaint (Youngken, 1876); the Delawares made a tea from the flower of the Elder (Sambucus Canadensis) "hollow tree." Some Indian mothers, for hiccups put a little wild ginger on the Indian baby's tongue. Among the Hopi Indians, inhabitants of the Pueblos of New Mexico and Arizona, the leaves of a plant named for the bat are placed on the head of a restless child to induce it to sleep in the daytime, because that is the time the slothful bat sleeps. It is not often that the Hopi children require an application of bat-plant medicine, but even the best of children get fractions sometimes (Hough, Walter: The Hopi Indians, 1915). The Hopi believe that the wild plants are most valuable for healing and religious purposes. Bunches of dried herbs, roots, etc. hang from the ceiling beams

of every house, reminding one of the mysterious bundles of "yarbs" in a negro cabin, and as occassion requires, are made into teas and powders for all sorts of ills.

When all other remedies had failed among the Delawares an emergency medicine was sometimes made from scrapings of deer antler. During its preparation a prayer was offered to the animal from which the antler was obtained. Wi. Tapanoxwe, an Indian medicine man of Lenape lineage who spent some time with Gladys Tantaquidgeon, herself a descedant of the Delaware Indians and an enthnologist well versed in American aboriginal lore, related to her an incident in which a trial was made by means of this substance. His niece was preparing it for a small child who was dangerously ill. She dropped a small quantity into a cup of hot water, and observing it settle to the bottom, knew that the little patient would not recover, for this was considered of bad import. The medicine was administered as a tea, but the child died in a short time (Tantaquidgeon, 1942). Golden Aster (Chrysopsia mariana) roots made into a tea quieted infants and also acted as a tonic for sickly children. Among the Delawares of Canada, Nannyberry (Viburnum lentago) and Mullein leaves are steeped and the tea administered in a case of measles. Mertensia ciliata, the so-called "blue medicine" of the Cheyennes was thought to be useful in cases of smallpox and measles, drunk in the form of an infusion.

As among all other folk, primitive and modern, there were many and various superstitions and myths concerning the child. When a child of the Guaranis of South America took sick all the relatives had to stop eating any food that they thought might be harmful to the child. Among the many North American Indian tribes, the father lifted his newly born offspring to the sun as if he would consecrate the child to its protection. The sun itself was a deity or else served to represent a benevolent divinity, just as Apollo, the sun was a healing diety to the Greeks. Among these tribes, as with some South Americans, blowing tobacco smoke through the child's mouth, as well as the mouth of any sick person, was common for they ascribed a sympathetic power to it. At the birth of a child to an Indian

woman of the middlewest in the early nineteenth century, Hallet describes how a hawk, symbolic of birth, was drawn by the attending mid-wife from a pouch and dropped to the ground. If it lay breast up in the pose of a man who dies a natural death, that would be considered a good omen. If on the contrary the bird fell breast downward, that was the position of a man slain in battle or cut off untimely and the omen was bad. It meant the child's time on earth would be short (Hallet). Among the Ojibways the medicine men, or midi (a society of wizards), carried out a ceremonial consecration of the new-born during which they danced in the temple wigwam with their medicine bags under their arms accompanied by drums and calabashes while the infant lay in the center and its parents and relatives stood on the sides; at the end of this ceremony the father received from the medicine man mysterious charms and amulets which were to be useful to the infant in later life: for instance a carefully wrapped white powder, or else some roots tied with red strings, etc. Before giving a name to a baby, the medicine man was consulted; usually a bird, or an animal or some laudable attribute was chosen, after some favorable sign appeared in some way. This was usually at the time when the child went out to "make his medicine," and this became his patron saint as it were. Various deities and idols were held especially sacred to childhood and infancy. Among the Aztecs the Goddess of birth was called Itzcuinam or dogmother. At the first bath of the infant the midwives of the ancient Mexicans repeated many blessings; among others, they turned to the newborn with these words: "Take this water, for the goddess Chalchuihcurje is your mother." The moon and the waters were the mythical original mothers of the Indians of North America; the Chippewas, Ottawas, Takkalis, etc., both rendered aid to the mother at childbirth, the babe in its cradle as well as the youth and maiden in their amours.

Upon the birth of a Delaware child, as among other Aboriginal tribes, it was given a pet to serve as the guardian of its health. If there was sickness about, the Indians believed that it would be visited upon the dog instead of its little master, a hopeful belief in the transference of disease or dependence upon

a scapegoat widespread throughout all primitive folklore. A small bag containing charcoal was tied around the pet animal's neck by the child's parents. If the animal died, it was buried and the child was given another pet. However, if the child fell sick and died, a release ceremony was performed by placing a string of wampum about its neck, thus freeing the animal. Among the ancient Zapotecs and other tribes inhabiting the Isthmus of Tehuantepec in Mexico, when a mother was about to be confined, the relatives assembled in the hut and commenced to draw on the floor figures of different animals rubbing each one out as soon as it was completed. This continued until the moment of birth and the figure that then remained sketched upon the ground was called the child's *tona* or second self. When the child grew old enough he procured the animal that represented him and took care of it, as it was believed that health and existence were bound up with that of the animals, in fact that the death of both would occur simultaneously. Soon after the child was born the parents, accompanied by friends and relatives, carried it to the nearest water where it was immersed while at the same time they invoked the inhabitants of the water to extend their protection to the child. In like manner they afterwards prayed for the favor of the animals of the land. The Quiches of Central America, among others, continued to call in the sorcerer even in modern times to take the horoscope of the new born and to appeal to the gods in its behalf. He also gave the infant the name of some animal which became its guardian spirit for life. Esquemelin related that the natives of Belize Coast and adjacent islands carried the new-born infant to the temple where it was placed naked in a hole filled with ashes, exposed to the wild beasts and left there until a track of some animal was noticed in the ashes. This became patron to the child who was taught to offer it incense and invoke it for protection.

Parental affection is nowhere more strongly brought out than in lamentations for the dead among some of the lowest tribes of California Indians. Feeding the dead has been in practice among many primitive peoples. The mother, with some of the

Indian tribes of New Mexico, used to drop milk from her breast on the lips of her dead babe; and in many parts of the world we meet with the custom of placing food near the grave so that the spirits may not hunger, or of placing it in the grave or coffin so that on its way to the spirit-land the soul of the deceased may partake of some refreshment. Among the ancient natives of Venezuela, "infants who died a few days after their birth, were seated around the Tree of Milk, or Celestinal Tree, that distilled milk from the extremity of its branches." Of the Yokaia, Mr. Powers tells us "It is their custom to 'feed the spirits of the dead' for the space of one year, by going daily to places which they were accustomed to frequent while living, where they sprinkle pinole upon the ground. A Yokaia mother who has lost her babe goes every day for a year to some place where her little one played while alive, or to the spot where its body was burned, and milks her breasts into the air. This is accompanied by plaintive mourning and weeping and piteous calling upon her little one to return, and sometimes she sings a hoarse and melancholy chant, and dances with a wild ecstatic swaying of the body."

Of the Miwok the same authority says: "The squaws wander off into the forest, wringing their arms piteously, beating the air, with eyes upturned, and adjuring the departed one, whom they tenderly call 'dear child,' or 'dear cousin' (whether a relative or not) to return."

Of the Niskwalli Indians of the state of Washington, Dr. Gibbs observes: "They go out alone to some place a little distant from the lodge or camp, and in a loud, sobbing voice, repeat a sort of stereotyped formula as, for instance, a mother on the loss of her child:

'Ah seahb! shed-da bud-dah ah-ta-but! ad-de-dah!
Ah chief my child dead alas!'

When in dreams they see any of their deceased friends this lamentation is renewed."

Very beautiful and touching in the extreme is the conduct of the Kabinapek of California:

"A peculiarity of this tribe is the intense sorrow with which they mourn for their children when dead. Their grief is

immeasurable. They not only burn up everything that the baby ever touched, but everything that they possess, so that they absolutely begin life over again—naked as they were born, without an article of property left."

Besides the custom of "feeding the spirits of the dead" just mentioned there exists also among certain of the California Indians the practice of "whispering a message into the ear of dead."

Among the western Eskimos, "the mother who loses her nursling places the poor 'papoose' in a beautifully ornamented box which she fastens on her back and carries about with her for a long while. Often she takes the miserable mummy in her arms and makes it a kind of toilette, disinfecting it and removing the mouldiness" (Chamberlain).

Of the Doracho Indians of Central America, the following statement is made: "When a mother, who is still suckling her child, dies, the latter is placed alive upon her breast and burned with her, so that in the future life she may continue to suckle it with her own milk." Powers remarks concerning the Korusu (Patwin) Indians of California: "When a woman died, leaving her infant very young, the friends shook it to death in a skin or blanket. This was done even with a halfbreed child." Of the Nishinam Indians, the same authority informs us: "When a mother dies, leaving a very young infant, custom allows the relatives to destroy it. This is generally done by the grandmother, aunt, or other near relative, who holds the poor innocent in her arms, and while it is seeking the maternal fountain, presses it to her breast until it is smothered. We must not judge them too harshly for this. They knew nothing of bottle nurture, patent nipples, or any kind of milk whatever, other than the human." Among the Wintlun, also, young infants are known to have been buried when the mother died shortly after confinement.

The Eskimos, Letourneau informs us, were wont to bury the little child with its dead mother, for they believed that unless this were done, the mother herself would call from Killo, the other world, for the child she had borne (Chamberlain). A Kodiak mother, upon the death of a child, would retire for a

time from camp, living in a small hut or "kennel," as Lisiansky described it (Bancroft, 1882).

Concerning the burial rites of a child, Bishop Cammerhof's journal of a journey to Shamokin, Pennsylvania, in 1748 relates that Logan's wife, who was a Mohican and a relative of Mohican Abraham, one of the first Moravian converts among the Indians, reported that while away on the annual hunt in November, her little daughter died, being, as she informed him, bewitched by a Delaware sorcerer. She immediately returned home with the corpse and gave it a Christian burial in a coffin but in the coffin with the corpse the mother showed the presents she had placed there, viz.: a blanket, several pairs of moccasins, buckskin for new ones, needle and thread, a kettle, two hatchets, and flint, steel and tinder, so that on arrival in the new country, it could go at once to housekeeping. The coffin was beautifully painted and had a supply of bear's meat, Indian corn and a calabash. The mother sat by the grave and wept and sang "Wake up my child, arise and eat; for five days ye have tasted no food . . . this my child was killed by the sorcerer."

Among the Indians of Peru the death penalty was meted out to a baby when it was born if a child who was still nursing from the mother died during this pregnancy. They reasoned that the unborn babe was in some mysterious way the cause of the death of the nursing child. Among the ancient inhabitants of Guatemala, if a nursing infant died, the mother held her milk back in her breasts for four days and would not suckle any other child since she believed that because of the dead child some harm or disease might affect the living one. This form of respect for the dead was called "navitia," from nahui, four, referring to the four day period of restraint (Ploss, 1891). The ancient Mexicans believed that if a twin birth occurred one of the two children could bring an end to the life of one of its parents; therefore, one of the twins was killed as soon as it was born (Ploss, 1876). Motolinia and Torquemado are the authorities for this, but the custom could not have been very general, for it is said that in Tlascala, a nearby province, the mother assigned a breast to each of the twins.

While most of the Brazilian Indians did not burden their pregnant women with the heavy labors to which they were otherwise accustomed, the women among the Patagonians of South America, the Dacotaks and most other North American Indian tribes did not receive any special attention (Ploss, 1876). Indeed, the Cahuilla Indians in California believed it was best for an expectant mother to have plenty of work to do so that the child would be industrious and strong (Miller, 1928). In 1513 ordinances in Spain for San Domingo and Puerto Rico, as well as later for Mexico and Peru, with the aim of protecting the health of the Indians restricted the work of pregnant women and of children (Moll, 1944). Women were to be given lighter tasks during the later months of pregnancy, and for several years after the birth of the child. As with all uncivilized races, and until quite recently among even the best of obstetricians, a belief was maintained that a pregnant woman should not gaze at a deformed person or make fun of him as the maternal impression thus created might affect her child. Care was also taken to avoid being frightened by dogs and other animals.

In the islands of the Torres Straits, flesh of the sole-like flat fish is said to cause the child in the womb to develop weak eyes and a misshapen nose; the eating of another fish causes the flesh of the child to become wrinkled like that of the old; while the use of the octopus flesh will cause the child's hands, mouth and fingers to become malformed. A Thompson River Indian woman will not eat hare when pregnant for fear her child will be born with harelip. If she ate or even touched with her hand a porcupine, or anything killed by an eagle or hawk, her child would look and act like these. If she ate the so-called fool-hen or squirrel the child would be foolish or a cry-baby (Teit, James: The Thompson River Indians, Memoirs. Amer. Mus. Nat. Hist., v. 2, p. 303–304). Among the Lummi Indians of Northwest Washington the pregnant woman does not eat halibut, which it is believed, causes white blotches on the skin of the baby; steelhead salmon which causes weak ankles; trout, which produces harelip; nor the beaver, which nurtures an abnormally large head; shad or blue cod, which gives the child

convulsions; the sea-gull and crane, which make it a "cry-baby"; and deer meat which brings on absent-mindedness. If she desires a small baby she drinks juices but does not eat fats (Stern, 1924). The Guarani mothers are said to have watched their diet during pregnancy and to have fasted, so that the infant would be more inclined to suckle after birth. Even the husband is reported to have fasted. Among some mid-western tribes, to assure the birth of a son, the expectant mother ate the seeds of a water gourd. The Aztec mother believed that sleeping in the daytime would contort the child's face; approaching too near the fire or standing in the hot sun would parch the foetus. Eating tzictli or chicle, our modern chewing gum, was thought to harden the palate of the unborn child, and to make its gums thick so that it was unable to suck, and also to communicate to it a disease called netentzzoponiztli; neither must the edible earth, of which the Mexicans were very fond, be eaten by the mother lest the child should prove weak and sickly. Other things which the woman fancied were to be given her because any interferences with her caprices might be hurtful to her offspring.

Chandless, an explorer of the Amazon River, on one occasion could not induce two of his native porters to row more strenuously; one said that he had left his wife pregnant, the other that his wife was at home with an infant. Their actions had to be tempered if their young ones were not to be injured (Miller, 1928). In Texas, the Carancahua expectant father, a month before the child was born (perhaps even for a longer time), partook only of fish and fasted at long intervals. The belief existed that at that period any injury to the father could be transmitted to the infant, and moreover, any evil spirits acquired by ingestion of flesh containing the same, would forthwith seek lodgment in the infant body (Dyer, 1916). Some California tribes likewise subscribed to these beliefs. The conduct of the husband was supposed in some manner to affect the unborn child and he was consequently laid under certain restrictions, such as not being allowed to leave the house, or to eat fish and meat (Bancroft, 1882). Among many of the various tribes of South American Indians, among whom

the custom of couvade is common, the spouse had to limit himself to special diet during the pregnancy of his wife; he had to avoid the pleasures of meats and live only on fish and fruit. For instance, if a woman of the Manhee Indians of Brazil became pregnant the husband immediately began a rather strict fasting period that lasted till after the delivery of the child. It is possible that they did this on the same grounds as the ancient Peruvians of the Inca empire, among whom twin births or natural malformations were of evil portent, which the parents tried to prevent by fasting for a certain time. According to Brett the Caribs believed that during the pregnancy of his wife a father should be denied the pleasure of certain foods because the child might incur certain harm therefrom; if he were to eat of a certain small animal, his child would be thin; of a certain little fish, it would be blind; of the peccary, it would develop a trunk-like mouth; of a certain bird, it would become dumb. Of the Indians of British Guiana, Mr. Im Thurn says they believe that if the father should eat the flesh of the capybara, the child would have large protruding teeth like the animal, while if he should eat that of the labba, the child's skin would be spotted. Apparently there is also some idea that for the father to eat strong food, to wash, to smoke, to handle weapons would have the same result as if the new-born baby ate such food, washed, smoked, or played with edged tools. The connection between father and child, the author believes, is thought by these Indians to be much closer than that existing between the mother and her offspring. In Paraguay and other South American localities a strict diet was enjoined upon the father and other relatives when the baby was sick (Ploss, 1876).

The Hopi Indians placed a swift-running insect on the wrist of a male child in order to make him a good runner; while on the girl's wrist is placed the cocoon of a butterfly in order to strengthen the wrist for corn growing (Miller, 1928). The Sioux and Algonquins tied a small bow and arrow to the cradle of a son so that he might grow up to be a great hunter, while the daughter was presented with other toys; on the other hand, the Natchez laid their boys upon panther skins, the girls on

buffalo hides, in order to confer upon them the favorable attributes of these animals. James Adair, a British trader among the Southern Indians for forty years, beginning in 1735, stated that by the custom of the country in Louisiana, little boys were bedded on panther skins or buffalo skins in order that they might grow into men of spirit, while little girls were couched on fawn skins in order to acquire the submissive characteristics thought becoming to Indian femininity. If when a child was born his feet were set on a bear rug, it would give him courage. It was also believed by some that a child born during a storm would grow up to be a chief and if a child began to walk on the day of a new moon he would grow up to be a fast swimmer. The Guarani of South America presented his boy with a miniature sword, bow and arrow and thereby reminded him pointedly to practice with his arms when he should reach manhood and be brave against his enemies. Even among the ancient Mexicans the child received from its father, according to his trade, similar reminders of tools, arms, etc., but the girl got a little spindle or weaving instrument (Ploss, 1876). Such implements as these were made either in miniature or painted on the bodies of the children. Among the Otomis of Mexico if the child was a boy one of the old men took it in his arms and painted on its breast an axe or some implement of husbandry, on its forehead a feather and on the shoulders a bow and quiver; he then invoked for it the protection of the gods. If the child proved to be a girl, the same ceremony was observed with the exception that an old woman officiated and the figure of a flower was traced over the region of the heart, while on the palm of the right hand a spinning wheel was pictured and on the left a piece of wool, thus indicating the several duties of after life.

The connection between infant dentition and a mouse (or rat), so commonly believed in and mentioned by the folklore of European peoples, was not unusual among the tribes of many distant lands, ancient Mexico, New Zealand, etc. The ancient Mexican belief prescribed: a deciduous tooth must be laid in a mouse-hole or else the teeth will not grow any more (Ploss, 1876).

Grease obtained from a black hen is rubbed on the aching gums of teething infants by the Nanticokes. Another remedy is to roast the brains of a dead rabbit and rub a small amount on the gums. Another informant suspends it by a cord from her child's neck. A mole's foot inclosed in a small bag is worn also for the same purpose. The deciduous teeth of children are thrown into the fire. If a dog should swallow one of these teeth a dog's tooth will grow in the child's mouth. During the teething period, the present-day Delawares hang a little bag containing finely crushed charcoal about the child's neck. The latter chews upon it to ease his aching gums. A loose milk tooth is pulled out by means of a string, its removal allowing the permanent tooth to grow in straight. The extracted tooth is taken outdoors by the child and thrown in the direction of the east, with these words: "My tooth, I place down here. I know when you return I will feed you white beans."

An Indian child's nails are trimmed with a knife or scissors. Care is taken not to lose any of the nail trimmings, which are carried into the woods and left upon a stump. At the same time the following words are addressed to the owl: "Grandpa, I now give you these, your grandchild's nails, to make frames for your eyes." The Hares and Dog-ribs of Northwestern Canada do not cut the finger nails of female children until four years of age, in order that they may not prove lazy (Bancroft, 1882). Babies and small children are not allowed to cry at night, nor older children to play after dark, for fear that their faces will become misshapen.

Among the Eskimos, the mother, after the birth of her child, must observe a great number of regulations referring particularly to food and work. She is not allowed for a whole year to eat raw meat or a part of any animal killed by being shot through the heart. In Cumberland Sound she must not eat for five days anything except meat of an animal killed by her husband or by a boy on his first hunting expedition. This custom seems to be observed more strictly and for a longer time if the new-born child dies. The mother also must keep a little skin bag hung up near her, into which she puts a little of her food after each meal, having first put it up to her mouth. This

is called laying food up for the infant, although none is given it (Corlett, 1935).

While nursing a child, a Delaware Indian mother must refrain from intercourse with any man other than her husband so that the infant may not have diarrhea.

It was believed by the inhabitants of Peru that in the matter of wet-nursing there was a vast difference between negro and Indian women. All diets and all medicaments were, like all people, divided into two classes, the cold and the hot, the heating and the cooling. Black, for example was cooling. Swollen joints must always be bandaged in black. If one were ill of a fever it was a good thing to go to the country and drink the milk of a black cow. So it was that the milk of a negro woman was cooler and more refreshing than that of an Indian. At the birth of a child no perfume of any sort must be allowed in the room for perfume would almost certainly cause convulsions.

In the rites of the Nagualists there occurs a baptism by fire which was celebrated on the fourth day after birth of a child, during which time it was deemed essential to keep the fire burning in the house, but not to permit any of it to be carried out, as that would bring bad luck to the child. This baptism of fire or purification by fire was in practice among ancient Aztecs of Mexico. To the second water baptism was added the fire baptism wherein the child was drawn through the fire four times. Among the Tarahumari Indians of the Mexican Sierra Madre, the medicine man "cures" the infant, so that it may become strong and healthy and live a long life. A big fire of corn cobs, or the branches of the mountain cedar, is made outside the house and the baby is carried over the smoke three times toward each cardinal point, and also three times backward. The motion is first toward the east, then toward the west, then south, then north. With a fire brand the medicine man makes three crosses on the child's forehead if it is a boy, four, if a girl. Among the Sacs and Foxes, North American tribes, after the delivery of a child the placenta was wrapped in the blanket on which delivery took place and secured to a tree to keep it from wild animals. Should a wolf or coyote

eat it, it was believed that the child would resemble such an animal and eventually be devoured by it. The placenta must not be thrown into the river lest the child should resemble a fish, or be drowned and eaten by a fish (Currier, 1891). "When a living nursing child is taken out at night, the mother takes a pinch of white ashes and rubs it on the face of the child so that the spirits will not trouble, because they say that a child still continues to hold intercourse with the spirit-world whence it so recently came" (Chamberlain).

The Delaware Indians of today, to cure a child from slobbering, catch a number of minnows and have the child hold them in his mouth. This is repeated several times a week until a cure is affected. The Nanticokes believe that if an excess of saliva runs from a baby's mouth, one should empty a thimbleful of water into the tea kettle when the child is nine days old. That will prevent the child from slobbering. They also believe that after severing the umbilical cord of an infant, it should be disposed of by burning, lest misfortune ensue. If the cord is dropped, the baby subsequently will be unable to retain its urine. To cure a child of bed-wetting, they feed him portions of a roasted "timber rat." This is believed to effect a sure cure. Wistapanoxwe and a companion once killed and roasted such a rodent and both agreed that the flesh was palatable and not unlike that of squirrel. They were reticent, however, as to the therapeutic effect. A white-footed mouse was used for the same purpose among the Gayhead Indians. When a child's hair was cut it had to be left in some out of the way place, for it was believed that if the birds used it in building their nests, the child would suffer from headaches. If the remnants were burnt it would cause the hair to become brittle. In northern Mexico it was a common custom to hang a small bag containing poisonous herbs round the neck of a child, as a talisman against diseases or attacks by wild beasts. The neighboring Mosquito tribes further to the south, just before the mother and child returned to the village from the seclusion of the maternity hut, fastened about the neck of the new arrival a "pew" or charm consisting of a bag of small seeds with which to pay old Charon

for ferriage across the river in case of an early death (Bancroft, 1882).

Indians of all ages had great faith in charms of various sorts. Little deerskin charms containing the dried fragments of the umbilical cord and tied to the clothing of children up to the age of six or seven years often had the shape of a lizard or turtle. This charm had wide currency and was common to the Sioux, Cheyennes, and Arapahoes. Mackenzie in the early nineteenth century observed the same thing among the Chipewyans in the north. He said: "The women have a singular custom of cutting off of a small piece of the navel string of new-born children which they hang about the neck. They enclose it in a case made of buckskin which they decorate with porcupine quills and beads."

A Cheyenne or Blackfoot woman might kill a newt, waterdog or mud-puppy and cause her little child to put its bare foot on the animal in order to cure leg ache. Sometimes with the same purpose in mind the figure of a lizard or newt might be sketched with charcoal on the sole of the foot (Grinnell, 1923).

REFERENCES

Andrews, E.: The Aboriginal Physicians of Michigan, Victor C. Vaughan Memorial Volume, Ann Arbor, 1903, p. 42.

Bancroft, H. H.: Works, San Francisco, 1882.

Bard, C. L.: History of Medicine in Southern California, Ventura, 1894.

Brinton, D. G.: Nagualism, Proc. Am. Philosophical Society, v. 33, 1894.

Chamberlain, A. F.: The Child and Childhood in Folk-Thought.

Charlton, M.: History of Medicine in Lower Canada, Ann. M. Hist., v. 5, 1923.

Corlett, W. T.: The Medicine-Man of the American Indian and His Cultural Background, Baltimore, C. C. Thomas, 1935.

Currier, A. F.: Reproduction in American Indian Women, Med. News, 58:390, 1891.

Dankers, J., and Sluyter, P.: Journal of a Voyage to New York and a Tour in Several of the American Colonies in 1679–1680, Memoirs of the Long Island Historical Society, v. 1, 1867.

Darby, G. E.: Indian medicine in British Columbia, Canad. M.A.J., p. 433, 1933.

Driscoll, T. L.: Early Therapy in Virginia, Centaur, 38:429, 1933.

Dyer, I. O.: Customs of Wild Tribes Near Galveston (Tex.), 1916.

Engelmann, G. J.: Labor Among Primitive People, ed. 3, St. Louis, J. H. Chambers & Company, 1884.

Essays and Observations, Philosophical Society of Edinburgh, 3:145, 1771.

Ettwein, J.: Notes of Travel, 1772, Penn. Mag. of Hist. and Biog., 25:218, 1901.

Flores, F. A.; cited by Cerna, D.: Historia de la Medicina en Mexico, Univ. Med. Mag., 4:740, 1891.

Foote, J. A.: Rickets prior to 1650, Am. J. Dis. Child., 34:443, 1927.

Gates, W.: The De La Cruz-Badiano Aztec Herbal of 1552, Maya Society, Baltimore, 1933, p. 111.

Hallet, R. M.: Michael Beam.

Harris, H.: California's Medical Story, San Francisco, 1932.

Hazard, S.: Register of Pennsylvania, Philadelphia, 1830.

Heagerty, J. J.: Four centuries of Medical History in Canada, Toronto, The Macmillan Company of Canada, Limited, 1928.

Heckewelder, J.: History, Manners and Customs of the Indian Nations Who Once Inhabited Pennsylvania and the Neighboring States, Memoirs of the Historical Society of Pennsylvania, v. 12, 1876.

Hodge, F. W.: Works, v. 1 (The Native Races), San Francisco, 1882.

Humboldt, Alexander von: Personal Narratives 1799–1804, New York, 1895.

Johnson, A.: Geographia America, by Peter Limdestroem, Philadelphia, 1925.

Kalm, P.: Travels Into North America, trans. by John Reinhold Forster, London, 1812.

Loomis, C. G.: Lapidary medicine, Bull. Hist. Med., 16:322, 1944.

Myers, A. C.: Narratives of Early Pennsylvania, New Jersey and Delaware, New York, 1912.

Miller, N.: The Child in Primitive Society, New York, 1928.

Moll, A. A.: Aesculapius in Latin America, Philadelphia and London, W. B. Saunders Company, 1944.

Monardes, N.: Joyfull Newes Out of the Newe Founde Worlde, Eng. ed. by John Frampton, London, 1925.

Moodie, R. L.: Antiquity of Disease, University of Chicago Press, Chicago, 1923.

Mooney, J., and Olbrechts, F. M.: The Swimmer Manuscript Cherokee Sacred Formulas and Medicinal Prescriptions, Smithsonian Institution Bulletin, No. 99, 1932.

Newburger, M.: Essays in the History of Medicine, New York, 1930, p. 39

Nuttal, Z.: Sorcery, Medicine and Surgery in Ancient Mexico, Bull. Johns Hopkins Hosp., 12:87, 1902.

Ploss, H.: Das Kind im Brauch und Sitte der Volker, Stutgart, 1876.

Ploss, H., and Bartels, M.: Das Weib im der Natür und Volkerkunde, ed. 3, Leipzig, 1891.

Patten, N. van: Medical Literature of Mexico and Central America; Papers of the Biblographical Society of America, 1930, v. 24.

Publications of the Colonial Society of Massachusetts, 1913, v. 14.

Radbill, S. X.: John Jones, Phisition; Bull. of the Inst. of the Hist. of Med., 6:145, 1938.

Rush, B.: Medical Inquiries and Observations, ed. 2, Philadelphia, 1805, 4 vols.

Scalpel, The, New York, 9:97, 1858.

Stern, B. J.: The Lummi Indians of Northwest Washington, New York, 1924.

Stone, E.: Medicine among the Iriquois, Ann. M. Hist., n.s., 6:529, 1935.

Stone, E.: Medicine Among the American Indians, New York, Paul B. Hoeber, Inc., 1932.

Tantaquidgeon, G.: Study of Delaware Indian Medicine and Folk Beliefs, Pennsylvania Historical Commission, Harrisburg, 1942.

Wild Babies, Harper's New Monthly Mag., November, 1878, p. 829.

Youngken, K. W.: Drugs of the North American Indian, Popular Science Lectures, Philadelphia College of Pharmacy, 2:213, 1923.

NUTRITIONAL REQUIREMENTS OF *DROSOPHILA HYDEI**

EDITH JU-HWA CHU†

The early work of Guyénot (1913), Northrop (1917), and Baumberger (1917, 1919) demonstrated that a yeast was necessary for growth of *Drosophila melanogaster*. Baumberger explained the nutritional value of yeast in terms of its protein content. Later, when vitamins and other growth factors were gradually discovered, Bacot and Harden (1922) assumed that vitamin B present in yeast was required by *Drosophila melanogaster* for normal growth. Due to rapid development of the chemistry of B vitamins van't Hoog (1935, 1936) was able to demonstrate that thiamin and riboflavin in yeast were essential for normal development of *Drosophila*, and also a sterol, such as cholesterol, was necessary.

Furthermore it was shown that yeast or liver fraction was still required in addition to other known requirements. Trager and Subbarow (1938) treated liver extract with barium hydroxide and alcohol and demonstrated that both fractions were essential for growth of *Drosophila*, while Lafon (1937) indicated there were two factors in yeast which were required by *Drosophila melanogaster* for normal growth, one being easily adsorbed and inactivated by hydrolysis and the other being neither adsorbed nor inactivated by hydrolysis. Recently Tatum (1939, 1941) demonstrated that *Drosophila melanogaster* larvae, grown under aseptic conditions on an agar medium containing amino acids, carbohydrate, salts, and certain vitamins, required in addition, for normal growth and complete development to the adult state, three fractions obtainable from yeast, namely fraction I, water and alcohol insoluble (residue from yeast autolysate); fraction II, water and alcohol soluble;

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and fraction III, water soluble and alcohol insoluble and also precipitated by barium hydroxide. Tatum identified the fraction II with a combination of nicotinic acid, vitamin B_1, vitamin B_6, and pantothenic acid. Wagner (1944) isolated a number of yeasts and bacteria from cactus fruits which were demonstrated to be essential for development of larvae of *Drosophila mulleri* and *Drosophila aldrichi*. It therefore appears that Drosophila is like the insects studied by Fraenkel (1943) in that yeast is necessary for growth.

We have undertaken an investigation of the nutritional requirements of *Drosophila hydei* with the prospect that some unknown growth factors might be identified in biological materials, particularly Brewers' yeast. It has been demonstrated that *Drosophila hydei* larvae, grown under aseptic conditions, require a factor or factors in yeast residue in addition to an agar medium containing amino acids, carbohydrate (glucose or sucrose), ergosterol or cholesterol, choline, pyrimidine and purine bases, inorganic salts, and a supplement of B vitamins (B_1, B_2, niacin, B_6 pantothenic acid, *p*-aminobenzoic acid, inositol, biotin, and folic acid). A number of pure compounds and biological materials have been tested for the active factor or factors. None of the pure compounds thus far tested is active, while some biological materials are active in less degree than the yeast residue. Attempts to treat the yeast residue by various means in order to get the active factor or factors in soluble form have not been very successful. Digestion of yeast, however, with trypsin or fresh liver seems promising for further investigation.

Experimental

The methods used by Wagner (1944) for sterilization of *Drosophila* eggs and transfer to an aseptic agar medium have been followed with minor changes. Flies two weeks old were transferred into a tube closed at either end by corks supporting glass cups in which food was placed. The food was freshly prepared with the following composition: corn starch, 5 gm.; glucose, 2 gm.; banana, approximately 1/8; H_2O, 25 ml. At the

end of 12–16 hrs., the flies were removed and eggs were transferred by means of a wire loop to a watch glass containing distilled water, and then into another watch glass containing 85% alcohol* enclosed in a petri dish and allowed to stand there about 30 min. with occasional stirring. Then the eggs were removed with a wire loop and placed on the agar medium as described below. Approximately 5 eggs were placed in each tube. The tubes were kept in an air incubator at 27° C. For several preliminary tests larval lengths were measured with an ocular micrometer day by day after hatching. Later, it was found that measurements of larval lengths on the fifth day (one or two days before pupation for normally developed larvae) are sufficiently accurate to compare activities of substances tested. The duration of the larval period or the time at which pupation occurs is another good criterion.

The aseptic agar medium in each tube has the following composition in addition to the substances tested (pH, 7–7.2):

agar	0.075 gm.	1-tryptophane	0.19 mg.
casamic acids	0.076 gm.	cysteine hydrochloride	0.19 mg.
sucrose	0.019 gm.	ergosterol	0.076 mg.
choline hydrochloride	0.116 mg.	thiamine hydrochloride	150 γ
guanine sulfate	0.274 mg.	riboflavin	150 γ
adenine hydrochloride	0.274 mg.	niacin	150 γ
uracil	0.274 mg.	pyridoxine hydrochloride	150 γ
K_2HPO_4	3.8 mg.	calcium pantothenate	150 γ
KH_2PO_4	3.8 mg.	*p*-aminobenzoic acid	150 γ
$MgSo_4 \cdot 7\ H_2O$	1.52 mg.	inositol	1500 γ
NaCl	0.076 mg.	folic acid	0.075 mg.u.†
$FeSo_4 \cdot 7\ H_2O$	0.076 mg.	biotin	0.18 γ
$MnSO_4 \cdot 4\ H_2O$	0.076 mg.		

The substances tested can be arranged in three groups, namely pure chemical compounds (Group I), natural materials (Group II), and natural materials after being treated (Group III).

*The 85% alcohol was used instead of 80% in order to compensate for the trace of water carried by the eggs.

†Potency 4200. See Mitchell, Snell, and Williams (1944).

Group I:

Substances	wt. per tube	larval length mm.	larval period
basal medium alone		0.9–1.5	dead after 14–18 days
folic acid	110 mg.u.*	1.0	dead after 18 days
biotin	1.25 γ	1.0	dead after 17 days
calcium pantothenate	0.125 mg.	1.0	dead after 19 days
inositol	0.625 mg.	1.1	dead after 17 days
p-aminobenzoic acid	0.125 mg.	1.0	dead after 16 days
niacin	0.125 mg.	1.1	dead after 16 days
vitamin B_6	0.625 γ	1.1	dead after 13 days
pyridoxamine	1.25 mg.	0.9	dead after 10 days
pyridoxamine and pyridoxal	100 γ each	0.8	.....
vitamins A_2 and D_3	0.125 mg. each	1.0	dead after 18 days
ascorbic acid, a-tocopherol, and menadione	0.125 mg. each	0.9	dead after 18 days
d-lactose	0.0625 gm.	1.0	dead after 19 days
d-mannose	0.0625 gm.	1.1	dead after 17 days
sucrose	0.0625 gm.	1.0	dead after 20 days
d-glucose	0.0625 gm.	1.0	dead after 18 days
d-fructose	0.0625 gm.	1.0	dead after 21 days
l-arabinose	0.113 gm.	0.9	.....
glucosamine	0.0625 gm.	0.7	.....
l-tryptophane	0.25 mg.	1.1	dead after 17 days
l-asparagine	0.11 gm.	0.9	dead after 16 days
glutamine	0.625 mg.	1.2	dead after 12 days
mixture of amino acids†	1.25 mg. each	1.0	dead after 12 days
linoleic acid	1.25 mg.	0.9	dead after 25 days
lecithin	0.13 mg.	0.8	dead after 14 days
sodium acetate	0.0556 gm.	0.8	dead after 14 days
cocarboxylase		0.8	.....
adenosine	0.25 mg.	0.9	.....
adenosine 5-phosphate	0.25 mg.	0.8	.....
adenosine 3-phosphate	0.25 mg.	no eggs hatched	.....
coenzyme I (1002)	0.25 mg.	1.2	.....
coenzyme I (502)	0.25 mg.	0.9	.....
guanaxine	0.125 mg.	0.8	.....
barium salt of inosinic acid	0.25 mg.	1.0	.....

*Potency 4200. See Mitchell, Snell, and Williams (1944).

†Amino acids: dl-isoleucine, dl-norleucine, l-leucine, dl-methionine, dl-threonine, 1(—)-proline, dl-phenylalanine, dl-norvaline, dl-serine, l-histidine. HCl, l-valine, d-glutamic acid, 1(—)-hydroxyproline, dl-alanine, 1(—)-tyrosine, 1(+)-lysine, d-arginine, 1(—)-tryptophane, l-aspartic acid, glycine, d-phenylalanine, cysteine. HCl.

Group II:

Substances	wt. per tube	larval length mm.	larval period
basal medium alone		0.9–1.5	dead after 14–18 days
vitab	0.11 gm.	1.0	dead after 23 days
glycogen	0.045 gm.	1.0	dead after 19 days

Substances	wt. per tube	larval length mm.	larval period
corn starch	0.11 gm.	1.1–3.2	some dead after 19 days some pup'tn after 11 days
corn starch	0.175 gm.	3.2	pupation after 8 days
pure egg albumin	0.058 gm.	1.2	dead after 10 days
gliadin	0.11 gm.	1.3	dead after 25 days
malt extract broth	0.11 gm.	1.2	dead after 25 days
gelatin	0.11 gm.	1.0	dead after 19 days
Wilson's peptone	0.11 gm.	1.3	dead after 17 days
Difco peptone	0.11 gm.	1.3	dead after 10 days
grass juice	0.0625 gm.	2.2	pupation after 12–13 days
grass juice	0.113 gm.	2.1	pupation after 8 days
vitamin-free casein	0.11 gm.	2.1	pupation after 10 days
vitamin-free casein	0.055 gm.	3.2	pupation after 9 days
vitamin-free casein	0.0275 gm.	1.9	pupation after 12 days
vitamin-free casein	0.0138 gm.	1.7	pupation after 11 days
technical casein	0.113 gm.	3.2	pupation after 7 days
technical casein	0.0625 gm.	3.2	pupation after 7 days
defatted wheat germ	0.11 gm.	2.4	pupation after 8–11 days
defatted wheat germ	0.0625 gm.	1.9	pupation after 8–10 days
defatted wheat germ	0.0313 gm.	1.4	pupation after 15 days
defatted wheat germ	0.015 gm.	1.3	pupation after 22 days
Lederle liver concentrate	0.113 gm.	2.0	pupation after 8 days
Lederle liver concentrate	0.0625 gm.	2.3	pupation after 8 days
liver protein	0.11 gm.	3.5	pupation after 7 days
liver fraction B	0.11 gm.	2.1	pupation after 13 days
liver fraction B	0.055 gm.	1.9	pupation after 12 days
liver fraction B	0.0275 gm.	1.8	pupation after 14 days
liver fraction B	0.0138 gm.	1.3	.....
nucleoprotein	5 mg.	0.9	dead after 12 days
animal nucleic acid	0.113 gm.	0.7	.....
Eastman yeast nucleic acid	0.0625 gm.	1.0	dead after 9 days
yeast after fermentation	0.0625 gm.	1.1	.....
yeast after fermentation	0.025 gm.	1.5	dead after 7–9 days
Difco yeast extract	0.1875 gm.	1.9	dead after 6 days
Difco yeast extract	0.113 gm.	1.9	dead after 6 days
Difco yeast extract, cellu flour	0.11 gm. 0.11 gm.	2.0	.....
Difco yeast residue*	0.15 gm.	4.2	pupation after 9–10 days
Difco yeast residue	0.113 gm.	4.0	pupation after 8–9 days
dried Fleischmann's yeast	0.0625 gm.	3.0	pupation after 6–7 days
dried Fleischmann's yeast	0.025 gm.	2.1	pupation after 8 days
fresh Fleischmann's yeast juice		1.0	.....
Anheuser-Busch No. 300 yeast	0.0625 gm.	3.3	pupation after 7–8 days
Anheuser-Busch No. 300 yeast	0.025 gm.	3.0	pupation after 7–9 days
Anheuser-Busch No. 200 yeast	0.11 gm.	4.3–5.0	pupation after 6–7 days
Anheuser-Busch No. 200 yeast	0.055 gm.	3.1–3.5	pupation after 8 days
Anheuser-Busch No. 200 yeast	0.0275 gm.	2.9–3.3	pupation after 8 days
Anheuser-Busch No. 200 yeast	0.0138 gm.	1.8–2.1	pupation after 11 days
Anheuser-Busch No. 200 yeast, albucid	0.113 gm. 0.025 gm.		
Anheuser-Busch No. 200 yeast, taurine	0.113 gm. 0.025 gm.	0.4	dead after 7 days

*Supplied by Difco Laboratories, Inc.

Group III:

Anheuser-Busch No. 200 yeast was treated by a number of different means and the soluble fractions and residues were separately tested.

(1) Autolysis.—Nine gm. of yeast was autolyzed at 37° C. for 24 hrs. and centrifuged. The soluble fraction (Extract 1) was diluted to 40 ml. The residue (Residue 1) was dried and reserved for later use.

(2) Autoclaving.—Two samples of 1 gm. each of yeast in 10 ml. water were autoclaved under 15 lb. pressure at 248° F. for 5 hrs. and 12 hrs., respectively. After centrifuging, the extracts and residues (Extract 2 and Residue 2, 5 hrs. autoclaving; Extract 3 and Residue 3, 12 hrs. autoclaving) were tested individually. Recently Golberg and coworkers (1944) reported autoclaving destroyed the growth promoting factor of yeast for mosquito larvae.

(3) Extraction with acetone.—Residue 1 obtained from autolysis was extracted with acetone in a Soxhlet Extractor for 10 hrs. The acetone solution (Extract 4) was concentrated to 40 ml. Residue 4 weighed about 6.4 gm. after drying.

(4) Extraction with pyridine.—Five gm. of yeast was extracted with pyridine in a Soxhlet Extractor for 3 days. The dark brown extract was distilled under vacuum to remove pyridine. The solids remaining after distillation were a dark brown viscous mass, weighing 200 mg. (Extract 5). Residue 5 was washed with water and dried.

(5) Extraction with glacial acetic acid.—One gm. of yeast was refluxed with glacial acetic acid for 1 hr. Extract 6 (10 ml. in water) and Residue 6 were separated as usual. The extract was evaporated to remove acetic acid.

(6) Extraction with hydrochloric acid.—Residue 4 was refluxed with 50 ml. of 6 N HCl for 3 hrs. The extract was distilled under vacuum to remove HCl, neutralized with 10% NaOH solution, and diluted to 130 ml. (Extract 7). Residue 7

was thoroughly washed with water and dried at 105° C.; wt. 1.77 gm.

One gm. of yeast was refluxed with 20 ml. of 3 N HCl for 1 hr. Extract 8 (10 ml. water solution) and Residue 8 were separated as usual.

(7) Extraction with NaCl solution.—The yeast protein included peptone, nucleic acid, and nucleotide was prepared according to the procedure of Dirr and Decker (1943). Twenty gm. of yeast was suspended in a solution of 20 gm. NaCl in 180 ml. water and digested in a steam bath for 72 hrs. and centrifuged. The extract was diluted to 132 ml. (Extract 9) and the residue (Residue 9) was washed and dried. A sample of 6.6 ml. of Extract 9 was dialuzed to remove NaCl.

(8) Extraction with $(NH_4)_2HPO_4$ solution.—Two gm. of yeast in 10 ml. of 0.16 M $(NH_4)_2HPO_4$ was shaken at 27–30° C. for 3 hrs. and the extract (Extract 10) and the residue (Residue 10) were separated.

(9) Digestion with enzymes.—To 25 gm. of yeast suspended in 250 ml. water was added 12.5 ml. of 10% NaOH solution and the pH was adjusted to 8.2. A suspension of 0.125 gm. trypsin and a few ml. of benzene were added. It was digested at 37° C. for 48 hrs. Then with addition of another 0.125 gm. trypsin, the digestion was continued for another 48 hrs. It was steamed and centrifuged. The extract (Extract 11) was diluted to 205 ml. and the pH was adjusted to 7.0. Residue 11 was washed and dried; wt., 9.7 gm.

A sample of 20 gm. of Difco yeast residue was digested with a mixture of takadiastase and papain (20 mg. each, suspended in 2 ml. water with a little glycerol) at 37° C. for 24 hrs. The extract (Extract 12) and the residue (Residue 12) were separated as before, the former being diluted to 130 ml. and the latter being dried at 105° C.

The results of testing the above extracts and residues are listed in the following table:

Treatment	Extracts and Residues	Amount	Larval Length mm.	Larval Period
autolysis	Extract 1	0.53 ml.	1.9	dead after 18 days
5 hrs. autoclaving.....	Extract 2	2.5 ml.	1.0	.....
	Residue 2	0.25 gm.	3.2–3.9	.....
12 hrs. autoclaving.....	Extract 3	2.5 ml.	2.2	.....
	Residue 3	0.25 gm.	3.0	.....
acetone extraction	Extract 4	0.53 ml.	0.8	dead after 13 days
	Residue 4	0.085 gm.	3.7	pupation 12 days
	Extract 1, Extract 4, Residue 4, without B vitamins	0.53 ml. 0.53 ml. 0.058 gm.	4.6	pupation 9 days
pyridine extraction	Extract 5	20 mg.	0.9	.....
	Residue 5	0.1 gm.	2.1	.....
glacial acetic acid extraction	Extract 6	2.5 ml.	1.3	.....
6 N HCl extraction	Extract 7, without B vitamins	1.7 ml.	1.3	dead after 20 days
	Residue 7, without B vitamins	0.0236 gm.	1.1	dead after 15 days
	Extract 7, Residue 7, without B vitamins	1.7 ml. 0.0236 gm.	1.0	dead after 14 days
3 N HCl extraction....	Extract 8	2.5 ml.	1.0	.....
	Residue 8	0.2 gm.	1.0	.....
NaCl solution extraction.	Extract 9 (before dialysis)	6.6 ml.	0.6	.....
	Extract 9 (after dialysis)	6.6 ml.	1.1	.....
	Residue 9	0.125 gm.	1.0	.....
$(NH_4)_2HPO_4$ extraction.	Extract 10	2.5 ml.	0.9–2.8	
	Residue 10	0.25 gm.	4.3	
trypsin treatment	Extract 11	2 ml.	1.6–2.0	pupation 12–14 days
	Residue 11	0.0625 gm.	4.7	pupation 7 days
takadiastase and papain treatment	Extract 12	0.75 ml.	0.9	dead after 13 days
	Extract 12, Residue 12	0.75 ml. 0.11 gm.	1.7	dead after 25 days
	Extract 12, Residue 12, Difco extract	0.75 ml. 0.11 gm. 0.11 gm.	3.6	pupation 7 days

(10) Digestion with dehydrated fresh liver.—One gm. of yeast in 10 ml. water was digested with 5 mg. of dehydrated fresh liver at pH 3, 6, and 8, respectively, at 37° C. for 48 hrs. Then the extracts (Extracts 13, 14, and 15) and residues (Residues 13, 14, and 15) were separated. At the same time 5 mg. liver without yeast and 1 gm. yeast without liver were treated under the same conditions and used as controls. The results are listed below:

Amount of liver for each tube	Amount of yeast for each tube	pH	Extracts and Residues	Larval Length mm.	Larval Period
1.25 mg.	0.25 gm.	3	Extract 13	2.0	.....
			Residue 13	3.1	pupation
		6	Extract 14	1.8	pupation 16 days
			Residue 14	3.2	pupation 9 days
		8	Extract 15	1.7	.....
			Residue 15	4.0	pupation 9 days
0	0.25 gm.	3	Extract 16	1.2	.....
			Residue 16	3.4	pupation 8 days
		6	Extract 17	1.2	.....
			Residue 17	3.3	pupation 8 days
		8	Extract 18	1.4	.....
			Residue 18	3.8	pupation 8 days
1.25 mg.	0	3	Extract 19	0.9	.....
			Residue 19	0.9	.....
		6	Extract 20	0.9	.....
			Residue 20	1.0	.....
		8	Extract 21	1.1	.....
			Residue 21	1.2	.....

Another series of digestions were made with 1 gm. yeast and varying amounts of the dehydrated fresh liver at pH 6.8. The results are as follows:

mg. liver for each gm. yeast	Extracts and Residues	Larval Lengths mm.	Larval Period
10	Extract 22	1.0–1.3	.....
	Residue 22	4.0–5.8	pupation 6 days
50	Extract 23	1.9–4.0	pupation
	Residue 23	2.0–5.0	pupation
100	Extract 24	1.9–4.0	pupation
	Residue 24	2.7–5.7	pupation
200	Extract 25	2.8	pupation
	Residue 25	5.7	pupation
300	Extract 26	1.8	.....
	Residue 26	2.2	.....
500	Extract 27	1.5	
	Residue 27	3.5	pupation

(11) Digestion with dead larvae.—The larvae were removed from food, washed successively with water, 80% alcohol, and then ether. The yeast was digested with varying amounts of larvae at different pH under various conditions. The results are tabulated below:

(a) pH: 6.8, temperature: 37° C., time: 48 hrs.

mg. larvae per gm. yeast	Extracts and Residues	Larval Lengths mm.	Larval Period
100	Extract 28	0.8–1.5	.....
	Residue 28	2.3	pupation
200	Extract 29	2.8	pupation
	Residue 29	4.3	pupation
300	Extract 30	2.8	pupation
	Residue 30	3.0–3.6	pupation
500	Extract 31	2.8	pupation
	Residue 31	2.6	pupation
300	Extract 32	...	.....
(without addition of yeast)	Residue 32	1.6	.....

(b) pH: 5.9, temperature: 37° C., time: 48 hrs.

mg. larvae per gm. yeast	Extracts and Residues	Larval Lengths mm.	Larval Period
0	Extract 33	1.0	.....
	Residue 33	3.3	pupation
20	Extract 34	0.7	.....
	Residue 34	1.7	.....
50	Extract 35	1.0	.....
	Residue 35	4.3	pupation
100	Extract 36	0.7	.....
	Residue 36	2.7	.....
200	Extract 37	0.7	.....
	Residue 37	...	.....

(c) temperature: 27° C., time: 96 hrs.

pH	Extracts and Residues	Larval Lengths mm.	Larval Period
2	Extract 38	0.6	.....
	Residue 38	1.4	.....
3	Extract 39	0.7–0.8	.....
	Residue 39	2.1	pupation
4	Extract 40	0.7–1.2	.....
	Residue 40	1.5–2.3	pupation
5	Extract 41	0.6	.....
	Residue 41	1.3	.....
6	Extract 42	0.7	.....
	Residue 42	1.4	.....
7	Extract 43	0.7	.....
	Residue 43	1.6	.....
8	Extract 44	0.8	.....
	Residue 44	1.8	.....

Digestion with intestinal mucosa of mice.—At different pH the No. 200 yeast was digested with varying amounts of intestinal mucosa of mice at 37° C. for 48 hrs. Results are as follows:

mg. intestinal mucosa per gm. yeast	pH	Extracts and Residues	Larval Lengths mm.	Larval Period
50	3	Extract 45	0.7	.....
		Residue 45	...	.....
	6	Extract 46	0.6	.....
		Residue 46	1.4	.....
	8	Extract 47	0.7	.....
		Residue 47	1.3	.....
100	3	Extract 48	1.0	.....
		Residue 48	2.4	pupation
	6	Extract 49	0.7	.....
		Residue 49	1.7	.....
	8	Extract 50	0.7	.....
		Residue 50	1.7	.....
200	3	Extract 51	0.7–1.1	.....
		Residue 51	1.8–2.3	pupation
	6	Extract 52	0.6	.....
		Residue 52	1.2	.....
	8	Extract 53	0.7	.....
		Residue 53	1.6	.....

Discussion

Experimental results have shown that a synthetic diet containing amino acids, glucose or sucrose, ergosterol or cholesterol, choline, pyrimidine and purine bases, inorganic salts, and a supplement of known B vitamins is not sufficient for growth of *Drosophila hydei* larvae and their subsequent development to the adult stage. None of the pure compounds listed under Group 1 promoted growth. The fact that *Drosophila hydei* do not need linoleic acid, vitamin E, and other fat-soluble factors is consistent with the finding of Fraenkel and Blewett (1945) using other insects. Among natural materials tested, whole yeast is best for promotion of larval growth and subsequent pupation, and casein, wheat germ, liver fraction B, liver protein, grass juice, and corn starch are also active, although in lesser degree. Taurine is toxic to development of larvae and albucid is inert in this respect.

Larvae grow better in the presence of yeast extract than of pure B vitamins, although they do not fully develop to adult stages. Therefore it is assumed that the yeast extract may still contain some unknown growth factor or factors.

The active factor or factors exist in the insoluble residue which is not extractable by water, acetone, pyridine, glacial acetic acid, or $(NH_4)_2HPO_4$ solution. The activity is destroyed by 3 N or 6 NHCl and also by NaCl solution, and weakened by long autoclaving at 248° F. under 15 lb. pressure. However, autoclaving of the medium for 10 min. has no weakening effect.

Digestion of yeast with takadiastase and papain gives an extract which is inactive, while the digestion with trypsin yields an extract which is active, although the activity is weaker than that of the yeast residue. This finding seems promising for further study.

Liver is known to contain a number of different enzymes. Digestion of yeast with dehydrated fresh liver yields an extract which is somewhat active. The best pH range for enzymatic digestion is between 6 and 8.

Since *Drosophila* larvae can digest yeast and utilize it for normal growth and development, it is thought that larvae

might contain some special enzymes accomplishing the function. But the experimental results have shown that dead larvae are unable to render the active material in soluble form. Neither has the digestion of yeast with intestinal mucosa been successful in bringing the active factor or factors into solution. Since digestion of yeast with dead larvae is unable to bring the active insoluble yeast residues into soluble form, it is assumed that either digestion of yeast by living larvae is not enzymatic in nature, or the enzymes necessary to digest yeast are only present in living larvae and are denatured when larvae are no longer living.

It has been demonstrated that the activity of yeast residue is not due to yeast nucleic acid.

Among the active natural substances tested, it is assumed that the nature of active factors may not be the same. For instance, the activity of liver fraction B is certainly due to some soluble factors and that of yeast residue to some insoluble factors. Whether they are identical with any of the following factors reported in the literature remains to be seen:

(1) Vitamin "M" factor in liver extract and yeast residue (Langston, *et al.*, 1938; Fournier Villada, 1942; Saslaw, *et al.*, 1943).

(2) Factors R and S described by Hill and coworkers (1944).

(3) Factor in dry yeast which cured pellagra-like conditions in guinea pigs as described by Solarino and Giorgio (1943).

(4) Unidentified factor(s) in yeast and liver essential to cure of achromotrichia in dogs on synthetic diets (Frost and Dann, 1944).

Summary

(1) A study of nutritional requirements of *Drosophila hydei* has been made. In addition to a basal medium containing amino acids, carbohydrate, ergosterol or cholesterol, choline, pyrimidine and purine bases, inorganic salts, and a supplement of B vitamins, a number of pure compounds have been tested for their effect on growth of *Drosophila hydei* larvae. None of them has been found essential or necessary.

(2) It has been demonstrated that factors in insoluble yeast residue are necessary for normal development of *Drosophila hydei*.

(3) As far as soluble growth factors are concerned, yeast autolysate or liver fraction B is superior to a mixture of all known B vitamins. In other words, there still exist in yeast extract and liver fraction B some unknown factors.

(4) The active factor or factors in yeast residue is not extractable by water, organic solvent or salt solutions, while the activity is destroyed by HCl and weakened by autoclaving.

(5) Digestion of yeast with trypsin or fresh liver seems promising for further study.

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BIBLIOGRAPHY

Bacot, A. W., and A. Harden: Vitamin Requirements of Drosophila. I. Vitamins B and C. Biochem. J., 16:148–152, 1922.

Baumberger, J. P.: The Food of Drosophila melanogaster Meigen. Proc. Natl. Acad. Sci., 3:122–126, 1917.

Baumberger, J. P.: A Nutritional Study of Insects with Special Reference to Microorganisms and Their Substrata. J. Exptl. Zool., 28:1–81, 1919.

Dirr, K., and P. Decker: The Value of Cultured Yeast in Human Nutrition. V. The Pure Protein Content of Yeast. Biochem. Z., 316:245–248, 1943; Chem. Abs., 38:4659, 1944.

Fournier Villada, R.: Vitamin M. Its Relationship to a Certain Syndrome of Diarrhea. Prensa méd. mex., 7:125–126, 1942; Chem. Abs., 37: 917, 1943.

Fraenkel, G.: Insect Nutrition. Sci. J. Roy. Coll. Sci., 13:59–69, 1943.

Fraenkel, G., and M. Blewett: Linoleic Acid, α-Tocopherol, and Other Fat-Soluble Substances As Nutritional Factors for Insects. Nature, 155:392–393, 1945.

Frost, D. V., and F. P. Dann: Unidentified Factor(s) in Yeast and Liver Essential to Cure of Achromotrichia in Dogs on Synthetic Diets. J. Nutrition, 27:355–362, 1944.

Golberg, L., B. DeMeillon, and M. Lavoipierre: Relation of "Folic Acid" to the Nutritional Requirements of the Mosquito Larva. Nature, 154: 608–610, 1944.

Guyénot, E.: Études Biologiques sur une Mouche, Drosophila ampelophila Löw. I. Possibilité de Ire Aseptique pour l'Individu et la Lignée. Compt. rend. Soc. biol., 74:97–99, 1913. II. Rôle des Levures dans l'Alimentation. Ibid., pp. 178–180. III. Changement de Milieu et Adaptation. Ibid., pp. 223–225. IV. Nutrition des Larves et Fécondité. Ibid., pp. 270–272. V. Nutrition des Adultes et Fécondité. Ibid., pp. 332–334. VI. Résorption des spermatozoïdes et Avortement des Oeufs. Ibid., pp. 389–391. VII. Le Déterminisme de la Ponte. Ibid., pp. 443–445.

Hill, F. W., L. C. Norris, and G. F. Heuser: Unidentified Vitamins Required by the Chick. J. Nutrition, 28:175–188, 1944.

Hoog, E. G. van't: Aseptic Culture of Insects in Vitamin Research. Z. Vitaminforsch., 4:300–324, 1935; Chem. Abs., 30:1097, 1936.

Hoog, E. G. van't: Aseptic Culture of Insects in Vitamin Research. Z. Vitaminforsch., 5:118–126, 1936; Chem. Abs., 30:5311, 1936.

Lafon, M.: Water-Soluble Factors Required by Drosophila. Compt. rend. Soc. Biol., 124:800–803, 1937; Chem. Abs., 31:5023, 1937.

Langston, W. C., W. J. Darby, C. F. Shukers, and P. L. Day: Nutritional Cytopenia (Vitamin M Deficiency) in the Monkey. J. Exptl. Med., 68:923–940, 1938.

Mitchell, H. K., E. E. Snell, and R. J. Williams: Folic Acid. I. Concentration from Spinach. J. Am. Chem. Soc., 66:267–268, 1944.

Northrop, J. H.: The Role of Yeast in the Nutrition of an Insect (Drosophila). J. Biol. Chem., 30:181–187, 1917.

Saslaw, S., H. E. Wilson, C. A. Doan, and J. L. Schwab: The "Vitamin M" Factor. Science, 97:514–515, 1943.

Solarino, G., and G. Giorgio: Does the Syndrome Occurring in Guinea Pigs on an Exclusive Corn Diet Depend on the Lack of a New Vitamin? Atti accad. Italia, Rend. classe sci. fis., mat. nat. /7/ 3:819–829, 1942; Chem. Zentr., 1943, II: 835–836; Chem. Abs., 38:5268, 1944.

Tatum, E. L.: Nutritional Requirements of Drosophila melanogaster. Proc. Natl. Acad. Sci., 25:490–497, 1939.

Tatum: E. L.: Vitamin B Requirements of Drosophila melanogaster. Proc. Natl. Acad. Sci., 27:193–197, 1941.

Trager, W., and Y. Subbarow: The Chemical Nature of Growth Factors Required by Mosquito Larvae. I. Riboflavin and Thiamine. Biol. Bull., 75:75–84, 1938.

Wagner, R. P.: The Nutrition of Drosophila mulleri and Drosophila aldrichi. Growth of the Larvae on Cactus Extract and the Microorganisms Found in Cactus. Univ. Texas Pub., 4445:104–128, 1944.

RENAL DAMAGE FROM FERROHEME PIGMENTS MYOGLOBIN, HEMOGLOBIN, HEMATIN

A. C. Corcoran, and Irvine H. Page*

Hemoglobinuria has long been recognized as a cause of renal injury. The properties of myoglobin (muscle hemoglobin), the pigment of red muscle, are similar to those of hemoglobin (Table I). Still, the association of myoglobin-uria with renal

Table I

Comparative Properties of Myoglobin and Hemoglobin (after Millikan)

Property	Myoglobin	Hemoglobin
Prosthetic group	Ferroheme	Ferroheme
Fe content	0.34 per cent	0.34 per cent
Combines reversibly with.....	O_2 CO_2	O_2 CO_2
Reversible redox activity.....	Fe'' Fe'''	Fe'' Fe'''
Dissociation curve	hyperbolic	sigmoid
Oxygen affinity	very high	moderate
Molecular weight	17,500	68,000
Ease of oxidation to "met-"..	very easy	less easy

damage was not generally recognized until 1940. Then patients whose limbs had been crushed in the debris of the London Blitz and who had recovered from the initial shock, were seen to pass into renal failure of varying and frequently lethal severity. This state was called "crush syndrome" (Bywaters and Beall).

The concern of this report is to assay the degree and nature of the renal injury which may result from myoglobinuria.

The prerequisites of hemoglobinuric renal damage (reviewed by Foy, Altmann, Barnes and Kondi) are not satisfactorily

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known. Still, it is generally recognized that renal injury does not follow in the wake of hemoglobinuria induced by injection of hemoglobin when the urine is neutral or alkaline (Baker and Dodds; DeGowin, Osterhagen and Andersch), while damage is common, although not invariable (de Navasquez) when the urine is acid. Similarly, myoglobinuria is harmless to renal function when the urine is not acidified (Yuile and Clark; Bing), but is harmful when the urine is acid (Bywaters and Stead). Since the urine is acid after crushing injury, this prerequisite of myoglobinuric renal damage is fulfilled in crush syndrome. Our study of the participation of myoglobin was therefore conducted in dogs whose urine was acidified by the administration of a diet rich in casein and meat and supplemented by daily administration of NaH_2PO_4 in a dosage sufficient to bring the urine to a level of pH 5 or 6.

Methods

Levels of renal function were determined in trained dogs with acid urine from the clearances of diodrast and inulin and the tubular secretory capacity for diodrast (Tm_D) by methods elsewhere described (Corcoran and Page). The values reported for these aspects of renal function, unless so noted, are the means of three periods of urine collection and clearance determination. The myoglobin injected in some of the experiments was prepared by Doctor P. J. Fouts by the method of Roche and Vieil; that used in other experiments was prepared by Doctor O. M. Helmer by the method of Theorell. The source material in both preparations was beef heart. No differences were found in the spectroscopic qualities or iron contents of the two preparations, which consisted almost wholly (95%) of myoglobin in meta- form. With the exception of one experiment, the first, the pigment was sterilized by Seitz filtration of a 0.9 per cent NaCl solution before injection. In two experiments, the metamyoglobin was reduced to a mixture of oxy- and reduced myoglobin by addition of ascorbic acid to the metamyoglobin solution before injection. The injections were made intravenously, usually over 10 or 15 minutes. The dosages selected were such as might correspond to the myoglobin

liberated in a human being with a crushed leg and thigh (Bywaters, Delory, Rimington and Smiles). Renal function was observed in control periods, during, and for an hour or more after injection, again at 48 or 72 hours and thereafter at intervals of one or two weeks or until a stable level of function was attained. The urine was colored by myoglobin for one or two hours after the injection. The amount of pigment excreted during this time was estimated by an adaptation of the "total hemoglobin" method of Evelyn and Malloy. The amount of pigment retained in the body was estimated by subtracting that excreted in 80 to 120 minutes from that injected. In many experiments the form in which the pigment was present in the urine was observed by a hand spectroscope.

For a comparison of myoglobinuric renal injury with that caused by hemoglobin, solutions of hemoglobin were prepared in 0.9 per cent NaCl from lysed dog red blood cells and injected intravenously in three experiments. Another aspect of pigmentary renal injury was examined by observations of the effect on renal function of the injection of hematin (sodium ferrihemate), prepared by the method of Drabkin and Austin. The recrystallized pigment was injected intravenously in solution brought to pH 7.4 (Morrison and Williams).

Results

The effects of injections of myoglobin and hemoglobin in aciduric dogs are summarized in Table II.

A. Myoglobin and metamyoglobin. Ten dogs were given metamyoglobin and a mixture of oxy- and reduced forms was injected in two. The one experiment in which the injected pigment solution was not sterile is included in Table II, but is excluded from further consideration because of the uncontrolled factor of bacterial invasion. This injection, made when the urine was nearly neutral, was followed by renal failure and death in uremia. This aspect of pigmentary injury will be the subject of a separate report.

Varying, sometimes minor degrees of renal injury followed the pigment injections in the other 11 experiments. The means of the functional changes are summarized in Figure I, from

TABLE II

Effects of Metamyoglobin, Myoglobin and Hemoglobin Injection on Renal Function in Dogs

Dog No.	Urine pH	Urine cc./min.	Injected mg./kg.	Retained mg./kg.	Percentage Normal Renal Function Immediate C_D	Immediate C_I	Immediate Tm_D	2 days C_D	2 days C_I	2 days Tm_D	2 weeks C_D	2 weeks C_I	2 weeks Tm_D
						Metamyoglobin							
21–93	6.5	1.2	88				9			11			4
15–67	6.0	0.8	78	21	76	59	74	78	57	29	76	77	66
15–79	5.6	0.7	64	14	101	64	61	72	80	80	97	93	90
15–62	5.8	0.1	146	114	7	8	7	24	18	17	74	66	80
16–38	5.6	0.2	189	39				113	119	90	94	117	86
16–54	5.8	0.2	215	65	142	105	48	81	95	61	88	74	96
15–86	5.3	0.4	208	78	118	52	27	123	80	66	86	77	66
16–74	5.8	1.0	252	97	138	107	30	104	103	58	136	80	67
16–04	5.1	1.2	100	25	142	107	94	85	84	58	93	92	74
15–66	5.9	0.8	101	37	71	45	34	78	61	35	81	82	72
						Myoglobin							
16–21	5.7	1.0	75	14	121	84	76	78	90	70	119	109	68
16–29	5.75	0.8	180	62	58	65	36	54	45	43	83	87	100
Mean	5.66	0.7	136	51	97	72	49	81	75	55	84	86	78
						Hemoglobin							
15–67	5.5	0.5	190		99	96	74	90	68	96	75	69	98
15–79	5.7	0.4	655		67	40	43	42	32	29	60	67	83
16–04	6.5	0.6	582		89	95	100	110	75	104	110	88	

Legend:

Urine pH and volume as measured at the time of pigment injection. Changes in renal function (C_D equals plasma diodrast clearance, C_I equals plasma inulin clearance, Tm_D equals tubular secretory capacity for diodrast) are expressed as per cent of control levels. The final observations of function were most commonly made at two weeks or more after pigment injection. The mean of metamyoglobin and myoglobin experiments is the arithmetic mean of the series, but does not include the experiment on Dog No. 21–93 in which the metamyoglobin given was not sterile.

which it is seen that diodrast clearance (the equivalent of renal plasma flow) is the function at first least affected and that tubular secretory capacity is that most depressed by pigment injection.

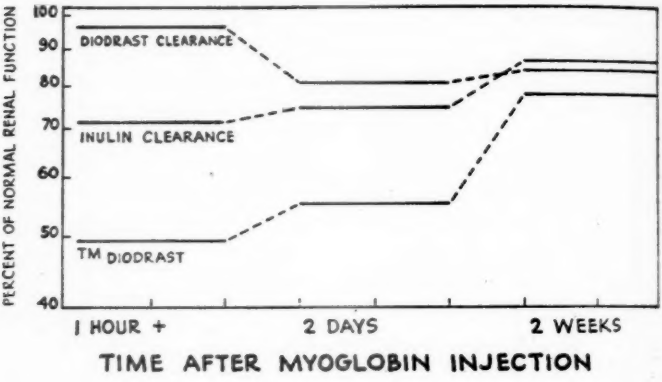

FIGURE I

The percentile changes in diodrast and inulin clearance and Tm diodrast following intravenous injection of myoglobin. Means of observations in eleven dogs.

The immediate effect of the injection was an increase in plasma diodrast clearance in 5 of 10 experiments. This change, attributed to renal hyperemia, was not associated with any elevation of body temperature, so that it is probably not the result of pyrogenic activity of the injected solution. Inulin clearance (glomerular filtration rate) was simultaneously depressed in 8 of 10 experiments and tubular secretory capacity diminished in 9. Two or three days after the injection, diodrast clearance had fallen below control levels in 8, inulin clearance in 9 and tubular secretory capacity in 10 of 11 observations. At a later date, averaging about two weeks after injection, the levels of renal function became stable. At this time, diodrast clearance remained depressed in 6, inulin clearance in 7 and tubular secretory capacity in 8 of 11 observations. The renal functional damage caused by injection of the ferrous forms

(oxy- and reduced myoglobin) was not different from that caused by metamyoglobin. This correspondence in the renal effects of the two pigments is attributed to the fact that, regardless whether myoglobin or metamyoglobin was injected, the pigment was present in the urine as a mixture of oxy- and meta-forms.

The courses of three experiments are presented graphically in Figure II. One of these (Dog No. 15–86) is selected because

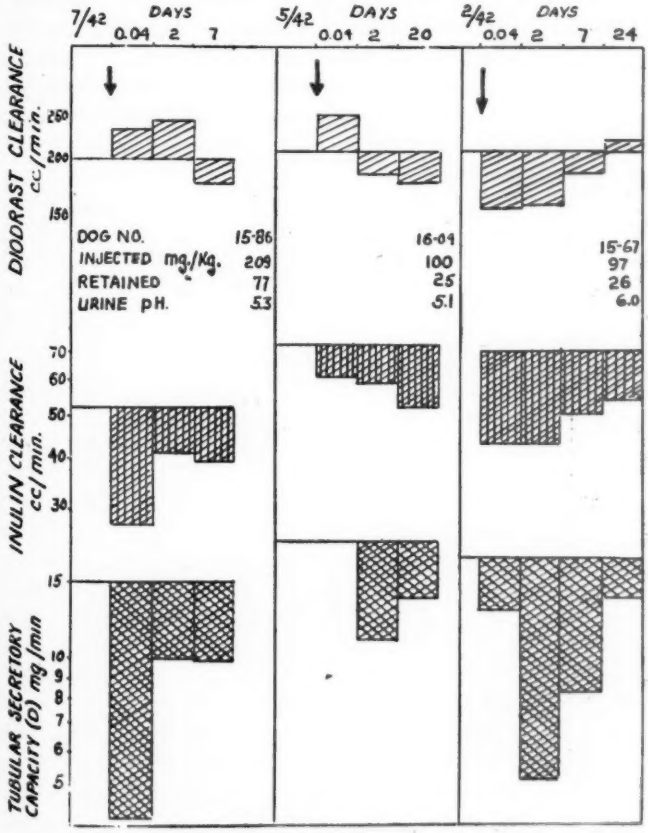

FIGURE II

Changes in renal function following injection of myoglobin in three dogs.

it illustrates a typical course while the other two are chosen to call attention to the fact, discussed below, that in these, as in one experiment with hemoglobin, the level of tubular secretory capacity was less at 48 hours after injection than it had been at a time when myoglobinuria had substantially ceased.

The proportion of the injected pigment retained is not a function of urinary pH in the range 5 to 6 (Figure III, inset). However, the degree of renal injury which follows the injection, as estimated from the percentile changes in tubular secretory capacity, is proportional to the amount of pigment retained (Figure III). This observation confirms that of Bywaters and

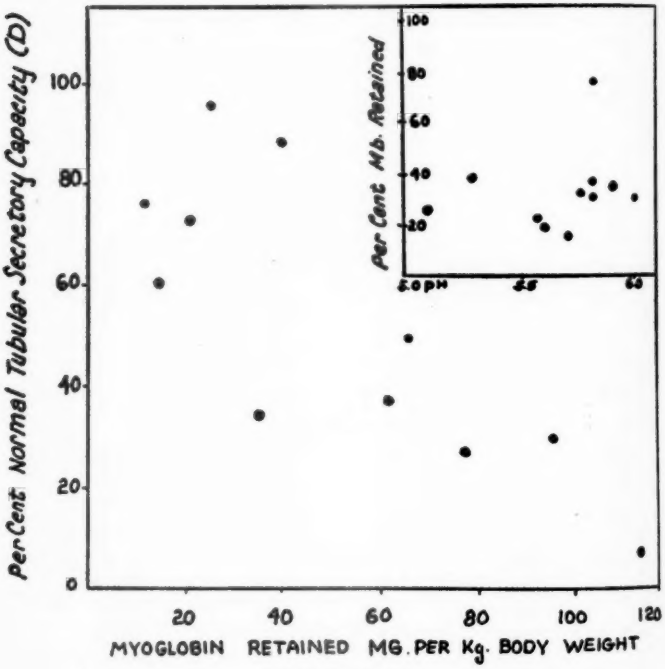

FIGURE III

Relation between tubular secretory capacity (ordinate) and retention (abscissa) in ten dogs. Inset: Absence of relation between myoglobin retention and urinary pH in the range pH 5.0 to 6.0.

Stead, who determined the azotemia which followed the injection of human metamyoglobin in aciduric rabbits.

B. Hemoglobin: The course of the renal injury in two experiments in which dog hemoglobin was injected was similar to that produced by myoglobin. No injury followed the injection of hemoglobin in one experiment when the urinary pH was 6.5. The differences in molecular size of hemoglobin and myoglobin are such that myoglobin is filtered through the glomerular capillaries into the renal tubules at about 25 times the rate of an equivalent plasma concentration of hemoglobin (Yuile and Clark). For this reason, the dosage of hemoglobin necessary to injure the kidney by intratubular deposition is greater than that of myoglobin.

C. Hematin. Hematin injected intravenously causes damage to many tissues, including the kidneys. Anderson, Morrison and Williams suggested that the basis of hemoglobinuric renal damage may lie in the intratubular liberation of this toxic ferriheme pigment. We, therefore, examined the effects of hematin injection with particular reference to the changes in renal function.

The renal effects of three injections at different levels of dose and rate of administration are shown in Table III. The smallest dose (14.5 mg. per kg. body weight) caused only a transitory renal hyperemia during and shortly after the injection. A larger dose (23.7 mg. per kg.) caused an immediate decrease in diodrast clearance and tubular secretory capacity, while inulin clearance was well maintained. This change was attributed to intense efferent arteriolar vasoconstriction with resultant increase in intraglomerular pressure; it was associated with only a slight increase in systemic arterial pressure. In succeeding days, the levels of diodrast and inulin clearances fell, although the depression on inulin clearance was the more severe. Tubular secretory capacity meanwhile slowly increased from the initial low level. The depression of inulin clearance at this time may be attributed either to afferent arteriolar constriction, or as seems more likely in view of the glomerulitis produced by hematin injection (Anderson, Morrison and Williams), to glomerular damage. The period of renal injury

TABLE III

Effects of Hematin on Renal Function

Dog No.		Clearances cc./min. Diodrast	Inulin	Filtration Fraction	Tm_D	Hematocrit	B.P. mm. Hg	Pulse
15–79	Control	203	62	0.31	12.8		106	
		210	60	0.28			118	112
		Hematin: 14.5 mg./kg. at rate of 0.28 mg./kg./min.						
	2 hour after-period (mean)	250	56	0.22	15.8		120	100
	2 days after	248	56.4	0.23	13.8		110	
15–67	Control	210	53	0.25	16.1		136	92
		194	55.6	0.32			114	88
		Hematin: 23.7 mg./kg. at rate of 0.49 mg./kg./min.						
	2 hour after period (mean)	142	51.6	0.37	10.3		144	104
	5 days*	101	24	0.24	11.7		142	
	8 days	142	30.8	0.217	12.5		136	
16–04	Control	192	75	0.39	14.0		138	100
		230	61	0.27		42	126	104
		Hematin: 32.6 mg./kg. at rate of 0.835 mg./kg./min.						
	Minutes							
	0–17 } Injection	211	55	0.26			128	108
	17–32 } period	16	0.5	0.03				
	32–43 }	2.5	0.1	0.04			130	96
	43–83† } After						70	88
	83–94 } period	2.05	0.08	0.04		29	66	98
	94–116 }	2.2	0.08	0.03		28	72†	96

*BUN on 5th day equals 53.6 mg. per 100 cc.
†Plasma hematin approximately 34 mg. per 100 cc. by iron analysis.

from hematin in this dog was marked by azotemia; the urine showed alternations of polyuria and hyposthenuria with oliguria and proteinuria for about 10 days. Bilirubin was present in the urine of the first 24 hours.

During the injection of the largest dose of hematin (32.6 mg. per kg.) renal function was unchanged at 17 minutes when one-third of the dose had been given. As the injection continued, inulin and diodrast clearances fell to very low levels and oliguria ensued. Arterial pressure and pulse rate were maintained at normal levels for several minutes after completing the injections, but arterial pressure later fell to 66 mm. Hg and one hour after the injection was 72 mm. Hg. At this time also, the animal did not respond with an increase of arterial pressure to a dose of angiotonin which caused a 20 mm. Hg increase in a normal dog of the same weight. The hematocrit index had decreased from 42 to 28. The appearance of the animal was that of shock. Death followed at about 12 hours after the injection. At autopsy, there were gross petechiae in the kidneys, liver, pancreas, adrenal, lung and mesentery. Microscopically, there was central hepatic necrosis and focal renal tubular necrosis with ischemia of glomeruli, but without pigment casts. The injection of hematin was found to inhibit blood clotting in each experiment, a fact which accounts for the hematomas which characterize its toxic action (Anderson, Morrison and Williams).

In summary, the reaction to intravenously injected hematin is one in which the dosage level of toxic action is highly critical. The effect, as regards the kidney, seems to begin with efferent vasoconstriction (Dog No. 15–67), which (Dog No. 16–04) spreads to afferent arterial tree and causes all but complete renal ischemia. The vasoconstriction may be followed by shock or, when present in milder degree, by moderate recoverable depression of tubular and glomerular functions.

Discussion

Intravenous infusion of myoglobin in conscious dogs with acid urine and in doses comparable to those liberated in crush syndrome, causes partially recoverable renal injury of varying

degree. Under the conditions of our experiments, the injury was not sufficient to lead to death in uremia, with the exception of an instance which differed in that the solution infused was presumed to be infected. Here an uremic course followed the injection of a relatively small dose of the infected pigment at a time when the urine was nearly neutral. It may be significant in regard to the effect of infected hemoglobin or myoglobin solutions that Theorell has recorded evidence that the physical state of myoglobin is altered by the products of bacteria, presumably as the result of proteolysis. Bywaters and Stead have recently reported the precipitation of fatal uremia in 4 of 27 aciduric (average urine pH 5.2) rabbits by the injection of sterile myoglobin in a dosage similar to that we have used, while varying degrees of renal injury (blood urea 100–860 mg. per cc.) were observed in 15 instances. It is therefore apparent that the excretion of myoglobin when the urine is acid may lead to renal injury. Since the urine pH may reach a level of 4.6 in clinical instances of crush syndrome, we conclude, with Bywaters and Stead that "myoglobin may play an important role in the genesis of renal failure" in this disorder. This conclusion we would qualify in view of our failure to obtain renal failure of uremic degree in the experiments here reported and in 20 normally hydrated, aciduric rats (urine pH 4.9 to 5.8), (unpublished observations) while the injection of similar doses of myoglobin in 90 dehydrated rats whose limbs had been crushed often results in severe renal damage (Corcoran and Page). Thus, where Bywaters and Stead place the emphasis entirely on aciduria, to this determinant of myoglobin-uric renal damage, we would add the other renal functional changes (oliguria, high urinary salt concentration, ischemia and functional renal tubular injury) which occur in hemorrhagic or tourniquet shock (Corcoran and Page; Corcoran, Taylor and Page).

The renal injury caused by myoglobin in our experience, as in that of Bywaters and Stead, is not proportional to the dose of pigment injected, but rather to the amount of the pigment retained in the body. Since nearly all the pigment is rapidly excreted when the urine is neutral or alkaline, it may

be assumed that the large amounts sometimes retained in aciduric animals are deposited in the kidneys. The nature of the injury here produced by hemoglobin or myoglobin is still in doubt. The sequence of events is often assumed to be as follows: (1) the pigment, present as a mixture of oxy- and metamyoglobin or as oxy-myoglobin, is filtered through the glomerular capillaries; (2) some fraction of the pigment is taken up by a sort of phagocytosis (Lison; Chambers) into the renal tubular cells; (3) the remainder, passing into the distal tubule, in which the urine may be acidified, forms methemoglobin or metamyoglobin in this region in the presence of acid tubular fluid. It may be significant that myoglobin, in comparison with hemoglobin, is rapidly oxidized to the ferric "meta" form. (4) Depending somewhat on the salt concentration, rate of fluid flow and on the pitch of aciduria reached in the distal tubule this is partially split to acid hematin. (5) Hematin is difficultly soluble at a low pH. It is therefore deposited and forms the nucleus of cast aggregation and tubular occlusion (Baker and Dodds, Bywaters and Stead). Such a view would be in accord with Bing's demonstration that methemoglobin is more injurious to renal function than oxymyoglobin, since injection of the "meta" advances the course of events by omitting step 3.

The injury produced by the precipitated mixture of hematin and globin might be wholly obstructive, wholly cytotoxic or a mixture of both. If it were wholly obstructive, then one would assume that tubular secretory capacity, inulin clearance (here accepted as equivalent to glomerular filtration rate) and plasma diodrast clearance (renal plasma flow) would be depressed in equal degrees. This, however, is not the case, for the function most affected is that of tubular secretory capacity while the depression of glomerular filtration ordinarily exceeds the damage to effective renal plasma flow. The disproportion between the changes in plasma flow and filtration rate is most evident immediately after the damage has occurred and disappears with the passage of time. Assuming, as seems likely,

that the decrease of filtration is dependent on tubular obstruction, then the initial maintenance of diodrast clearance is evidence of renal hyperemia.

That renal hyperemia is present is borne out by the increased diodrast clearances present in 5 of 10 observations. The occurrence of renal hyperemia during the injection of myoglobin or hemoglobin contrasts with oncometric observations (Mason and Mann, Hesse and Filatov) of decreased kidney volume during hemoglobin infusion and vitiates the supposition that hemoglobinuric renal injury is primarily ischemic. Later, as hyperemia subsides, inulin and diodrast clearances resume their normal ratio. Finally, when renal function is stabilized at some level of partial recovery, the clearances of the two substances are usually equally depressed below the control level. The residual damage at this time is taken to indicate continuing obstruction and degeneration of certain nephrons.

In so far as tubular secretory capacity, a proximal tubular function, is depressed in proportion to glomerular filtration, it may be assumed that the damage to both is the result of nephric obstruction. Damage to tubular secretion beyond the degree of glomerular hypofunction may be due to alteration in tubule cells by the space-filling vacuoles and reactive changes of proximal tubule cells incident to ingestion of a pigment such as myoglobin or hemoglobin (Chambers). That such a change may occur is suggested by (1) the disproportionate depression of tubular secretory capacity observed in the early phases of our experiments, (2) the fact that the depression of secretory capacity continued to advance in some experiments after the excretion of the pigment had ceased and (3) the known reactions of proximal convoluted tubules to the presence of intratubular hemoglobin. We may thus account for much of the injury incident to the coincidence of myoglobinuria and aciduria on the basis of a combination of distal tubular obstruction and, through excessive cellular ingestion of pigment, proximal tubular surfeit.

Micro-dissection of the kidneys in hemoglobinuric nephroses (Oliver) has established that a renal lobule which in histological section appears only partly obstructed by pigment casts, may

in fact be completely occluded since the casts form at different levels. Histological examination of such tissues has repeatedly shown the association of distal tubular proliferation and necrosis (Ayer and Gauld; Kimmelstiehl; Goldring and Graef) with peritubular interstitial edema, sometimes in the absence of obstructing casts. Consequently, many incline to the view that the renal injury of hemoglobinuric (and, by implication) of myoglobinuric renal injury represents a combination of obstructive and toxic distal tubular lesions. The attribution of hemoglobinuric renal injury to "allergy," although histologically tenable, does not account for the occurrence of such damage during the first injection of the pigment. Bywaters and Stead, who injected human myoglobin in rabbits, considered it possible that the injury might depend on the heterologous character of the injected pigment. Such a reservation would apply also to our experiments, but we believe it invalidated by the similarities of the renal functional changes after injection of either beef myoglobin or dog hemoglobin. The injury, therefore is to be attributed to the heme-globin pigment as such.

The experiments here reported, and those of Anderson, Morrison and Williams establish that, at a critical level of dosage, intravenously injected hematin is nephrotoxic. Of course, the renal injury from intravenously injected hematin necessarily differs from that which is caused by hemoglobin or myoglobin injection. This difference lies: (1) in the anatomical site at which hematin comes in contact with the cell, which in the case of myoglobinuria, is the tubular; while, after injection of hematin, it is the basilar aspect of the cell. (2) In the fact that intravenously injected hematin forms in close union with plasma albumin, a substance which, in unpublished observations, we have shown to be nontoxic to rats in large doses of ferro-heme. In human beings, this complex of hematin and albumin—which Fairley calls methemalbumin—has a characteristic absorption spectrum. The critical dosage level at which the toxicity of hematin appears is probably due to this protein-binding; a toxic action not being exerted until the plasma protein is saturated and free pigment present in the

plasma water. (3) The toxic action of hematin is further distinct from that of metamyoglobin in that it is accompanied by marked renal vasoconstriction and, in large doses, with a shock-like state. Nevertheless, the demonstration that injected hematin is nephrotoxic is sufficient grounds for the conclusion that liberation of hematin within the renal tubule, where the tubule fluid, unlike plasma, is not rich in pigment-binding protein, would injure the cells.

Apart from crush syndrome, myoglobinuria is known to occur as "Haff disease," a condition attributed to eating fish from a river polluted by residues from cellulose factories near Hamburg, as paralytic equine myoglobinuria and as a similar acute paralytic myoglobinuria in man. Eight probable cases of the latter condition have been reported, including that of Bywaters and Dible, whose patient died in renal failure. Myoglobinuria is therefore not a widespread clinical problem in days of peace, except as crush syndrome may occur in the accidents of civilian life. Hemoglobinuria is the more important problem. We believe that our observations, with the modifications noted in the discussion, apply equally to hemo- or myoglobinuria.

Summary and Conclusion

1. The injection of myoglobin in aciduric, conscious dogs results in varying degrees of renal functional impairment. Myoglobinuria may therefore be a significant factor in the renal injury of crush syndrome.

2. The degree of renal injury caused by myoglobin is proportional to the amount of the injected pigment retained in the body.

3. The function of tubular secretion of diodrast is most depressed, that of glomerular filtration (inulin clearance) in lesser degree, and renal plasma flow (diodrast clearance) least of all.

4. A similar injury may be obtained in aciduric dogs by the infusion of hemoglobin.

5. A form of renal injury, associated with intense renal vasoconstriction, but also characterized by toxic cellular changes,

may be produced by intravenous infusion of large amounts of hematin (sodium ferri-hemate).

6. The renal injury in aciduric, myoglobinuric dogs is attributed to (a) obstruction of tubules by pigment, (b) ingestion of the pigment by tubule cells, resulting in impaired tubular secretory activity and (c) cytotoxic distal tubular activity of intratubularly liberated hematin.

REFERENCES

Anderson, W. A. D., Morrison, D. B. and Williams, E. F., Jr.: Pathologic changes following injections of ferrihemate (hematin) in dogs. Arch. Path. 33:589, 1942.

Ayer, G. D. and Gauld, A. G.: Uremia following blood transfusion, the nature and the significance of the renal changes. Arch. Path. 33:513, 1942.

Baker, S. L., and Dodds, E. E.: Obstruction of the renal tubules during the excretion of haemoglobin. Brit. J. Exper. Path. 6:247–260, 1925.

Bing, R. J.: Effect of hemoglobin and related pigments on renal function of normal and acidotic dog. Bull. Johns Hopkins Hosp. 74:161–176, 1944.

Bywaters, E. G. L. and Beall, D.: Crush injuries with impairment of renal function. Brit. M. J. 1:427–432, 1940.

Bywaters, E. G. L., Delory, G. E., Rimington, C. and Smiles, J.: Myohaemoglobin in the urine of air raid casualties with crushing injury. Biochem. J. 35:1164–1168, 1941.

Bywaters, E. G. L. and Dible, J. H.: Acute paralytic myohaemoglobinuria in man. J. Path. and Bact. 55:7–15, 1943.

Bywaters, E. G. L. and Stead, J. K.: The production of renal failure following injection of solutions containing myohemoglobin. Quart. J. Exper. Physiol. 33:53, 1944.

Chambers, Robert: Personal communication to the authors.

Corcoran, A. C. and Page, Irvine H.: Effects of hypotension due to hemorrhage and of blood transfusion on renal function in dogs. J. Exper. Med. 78:205–224, 1943.

Corcoran, A. C. and Page, Irvine H.: Unpublished observations.

Corcoran, A. C., Taylor, R. D. and Page, Irvine H.: Immediate effects on renal function of the onset of shock due to partially occluding limb tourniquets. Ann. Surg. 118:871–876, 1943.

DeGowin, E. L., Osterhagen, H. F. and Andersch, M.: Renal insufficiency from blood transfusion; relation to urinary acidity. Arch. Int. Med. 59:432–444, 1937.

de Navasquez, S.: Excretion of haemoglobin with special reference to "transfusion" kidney. J. Path. & Bact. 51:413–425, 1940.

Drabkin, David L. and Austin, J. H.: Spectrophotometric studies: Hemochromogens. J. Biol. Chem. 112:89, 1935.

Evelyn, Kenneth A. and Malloy, H. T.: Microdetermination of oxyhemoglobin, methemoglobin, and sulfhemoglobin in a single sample of blood. J. Biol. Chem. 126:655–662, 1938.

Fairley, N. H.: Fate of extracorpuscular circulating haemoglobin. Brit. M. J. 2:213–217, 1940.

Foy, H., Altmann, A., Barnes, H. D. and Kondi, A.: Anuria, with special reference to renal failure in blackwater fever, incompatible transfusions, and crush injuries. Tr. Roy. Soc. Trop. Med. & Hyg. 36:197–238, 1943.

Goldring, W. and Graef, I.: Nephrosis with uremia following transfusion with incompatible blood; report of 7 cases with 3 deaths. Arch. Int. Med. 58:825–845, 1936.

Hesse, E. and Filatov, A.: Experimentelle untersuchungen über das Wesen des hämolytischen schocks bei Bluttransfusion und die therapeutische Bieinflussung desseben; die Nierenfunktionsstörungen in akuten Experiment. Ztschr. f. d. ges. exper. Med. 86:211–230, 1933.

Kimmelstiel, P.: Acute hematogenous interstitial nephritis. Am. J. Path. 14:737–761, 1938.

Lison, L.: Zur Frage der ausscheidung und speicherung des hamoglobins in der amphibienniere. Beitr. Path. Anat. Allg. Path. 101:94–108, 1938.

Mason, J. B. and Mann, F. C.: Effect of hemoglobin on volume of kidney. Am. J. Physiol. 98:181, 1931.

Millikan, G. A.: Muscle hemoglobin. Physiol. Rev. 19:503–523, 1939.

Morrison, Dempsie B. and Williams, E. F., Jr.: The solubility and titration of hemin and ferrihemic acid. J. Biol. Chem. 137:461, 1941.

Oliver, Jean: Personal communication to the authors.

Roche, Jean and Vieil, Helene: Preparation and molecular weight of crystalline horse myoglobin (muscular hemoglobin). Compt. rend. Soc. Biol. 210:314–316, 1940.

Theorell, A. H. T.: Kristallinisches myoglobin; kristalisieren und reinigung des myoglobins sowie vorlaufige mitteilung über sein molekulargewicht. Biochem. Ztschr. 252:1–7, 1932.

Yuile, C. L., and Clark, W. F.: Myohemoglobinuria, study of renal clearance of myohemoglobin in dogs. J. Exper. Med. 74:187, 1941.

RETICULO-ENDOTHELIAL IMMUNE SERUM (REIS):

IV. Experimental Production of Anemia and Bartonellosis in Rats by Inhibitory Dosage

LUDWIK ANIGSTEIN AND C. M. POMERAT*

It has been shown in previous communications that the reticulo-endothelial immune serum (REIS) when applied in strong concentrations has a definite inhibitory action on tissue grown *in vitro* (Pomerat and Anigstein, 1944, 1945). Limitation of outgrowth associated with cellular clumping was noted in explants of embryonic or post-embryonic spleens of rats, guinea pigs, and chicks when homologous REIS was used. These phenomena have induced us to analyze the biological properties of REIS *in vivo* inasmuch as its specific action on the reticulo-endothelial system and on the defense mechanism against various pathogens has been recently advocated by a group of Soviet scientists (Bogomolets and associates, 1943). The numerous conditions specified by Bogomolets include malignancy on which REIS is claimed to have a beneficial effect. While the mechanism of action of REIS is still obscure we may speculate that it may affect malignant tumors either by direct limitation of growth or by stimulating the defense mechanism of the host.

The purpose of the present study is to analyze some of the properties of REIS *in vivo*, particularly its action on the animal injected with relatively high dosage of the serum. In the search for a test object it was believed that the albino rat with its readily responding hemopoietic system and its high ratio of spleen to body weight would be particularly suitable for experiments in which the RES is involved. Moreover, it is known that the albino rat of the bartonella carrier stock reacts with

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spectacular blood changes to splenectomy and, under certain conditions, to other extragenous factors.

Materials and Methods

Animals used in this study were adult albino rats which, although originally of the Wistar stock, were bred in this institution for a number of years and when splenectomized proved to be bartonella carriers. For control experiments recently obtained rats of the Wistar strain were used. These bartonella-free rats were kept separate and all necessary precautions were taken to isolate them from possible infection. All animals were fed with Purina dog-chow and provided with drinking water. Prior to splenectomy or administration of the serum daily blood counts, hemoglobin content and smears were made from tail blood and continued for ten to seventeen days following such procedures.

The anti-sera were prepared according to technique advised by Marchuk (1943) for his ACS serum; namely, by repeated i.v. injections of rabbits with the slightly centrifuged suspensions of fresh spleens from albino rats. Four to five injections were usually given at four-day intervals. Before the removal of the spleen the rat was bled white in order to obtain this organ as blood free as possible. The ground spleen tissue was then suspended in saline in proportion 1:6. For the initial injection 0.5 cc. of the suspension was used, followed by an increase to 1.0 cc. Simultaneously the rabbits were given the original spleen moiety by mouth (1 cc. each time). In addition, spleen and bone marrow from baby chicks and from adult guinea pigs were used as antigens for the preparation of heterologous immune sera. The latter served in a series of control experiments for comparison of their action with that of organ and species specific sera. Finally, sera from normal rabbits were used for routine controls. The potency of the immune sera was determined by the complement fixation test. Fresh spleen or spleen and marrow were used as antigens according to the technique of Bogomolets (1943). Various titers were obtained from 1:200 to 1:1600. However, due to the anticomplementary

action of the antigen this method of titration could not always be carried out. In several instances the titration of the sera was performed by one of us (C.M.P.) in using tissue cultures and evaluating the serum action on the outgrowth of the explant. It is possible that this method will prove superior to the complement fixation test.

Of the total of 44 rats used in this study 8 were of the Wistar bartonella-free strain, the remainder being bartonella carriers. Splenectomy was performed in 6 rats of the carrier stock and in 3 of the Wistar rats. Sixteen bartonella carriers were injected with the homologous anti-rat spleen serum, 3 with anti-chick and 3 with anti-guinea pig serum. Seven Wistar rats were treated with homologous REIS. Six carrier rats were injected with normal rabbit serum. Single injections of 0.5–1.0 cc. of the serum were given intraabdominally in all the experiments.

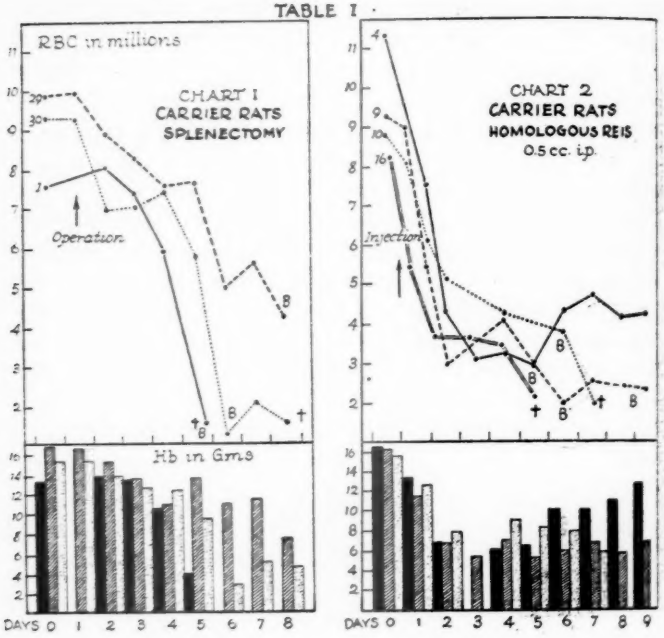
Results

A. Effect of Splenectomy

It is not the purpose of this study to present a detailed account of the effects of splenectomy on the bartonella carrier rat inasmuch as this subject has been extensively investigated by others. An outstanding presentation of the problem of anemias due to bartonella will be found in the monograph by Weinman (1944). In the present work the splenectomized rat was merely used as an object for comparison with the response of the unoperated rat to the REIS. In other words, attempts were made to simulate the surgical removal of the spleen with the use of this extragenous biological factor and to analyze its action and specificity.

The first signs of anemia were noted in bartonella carriers within 72–96 hours after splenectomy (Table I, Chart 1). Simultaneously bartonellae appeared in the red cells. This was followed by a precipitous drop in the erythrocyte count and in the hemoglobin content (Table I, Chart 1). Bartonellae appeared singly as well as in characteristic clusters within the intact erythrocytes and within the shadows of hemolyzed

TABLE I



corpuscles. As blood destruction progressed more numerous extracellular parasites were seen. The preparation of blood smears was occasionally difficult because of instantaneous coagulation—a phenomenon also noted by others (Weinman, 1944, p. 291). Of 6 splenectomized rats 4 died on the fifth and eighth day, while the remaining 2 recovered. The quantitative abnormalities of blood constituents were accompanied by prominent cellular changes. Large erythrocytes appeared simultaneously with the first signs of anemia, the number of macrocytes increasing with its severity. Normoblasts were usually present although few in number, whereas polychromatophilia and poikilocytosis were marked in every splenectomized rat during the anemia period.

Parallel to the changes in the red cells and hemoglobin there was a marked reaction of the white cells. The leucocytosis

noted by most investigators was a constant phenomenon. The increase in white cell count usually preceded the anemia, with a daily rise and usually reaching the peak on the fifth or sixth day. The highest figure of 76,600 white cells was attained in rat No. 30 on the fifth day after splenectomy. A marked neutrophilia dominated the picture with a steady increase of polymorphs reaching the peak of 85% and coinciding with the highest leucocytosis. These changes were previously noted (Weinman, 1944, p. 291).*

Splenectomy performed on 3 rats of the Wistar strain did not affect the erythrocytes except for slight daily variations within the range of 8–10 million cells. In one rat (No. 27) a drop from 11,000,000 to 6,170,000 was noted on the fifth day but a normal level was reached within the following 4 days (Table II, Chart 5). The same applied to the hemoglobin content. Total white counts are not available for this series but the differential count showed a relative neutrophilia beginning with the day following splenectomy and observed during the subsequent ten days. The highest level (54%) was reached by rat No. 27, otherwise the percentage of polymorphs fluctuated between 34 and 50. In no case were bartonellae noted.

B. Effect of Administration of REIS

Although the mechanism of the immune phenomena which regulate the latent bartonella infection in the carrier rat is still obscure, the spleen undoubtedly plays an important role in keeping the host and the parasite in balance. As has been repeatedly shown, the removal of the spleen is the most drastic method by which the disturbance of this equilibrium can be achieved, although a similar but milder effect was obtained by other extragenous agents, such as blockading with India ink and colloidal dyes. It was found also that a bartonellosis can be produced not only by blockading the RES of rats with an intact spleen but also by blockading splenectomized rats. According to Weinman (1944) this indicates "that the extrasplenic

*According to Weinman (1944, p. 291) normal values for the albino rat are: Red cells 9,200,000; Hb. 16.9 Gm.; white cells 11,590; neutrophils 27%; lymphocytes 67.9%; monocytes 5.3%; eos. 2.1%; bas. 0.77%.

TABLE II

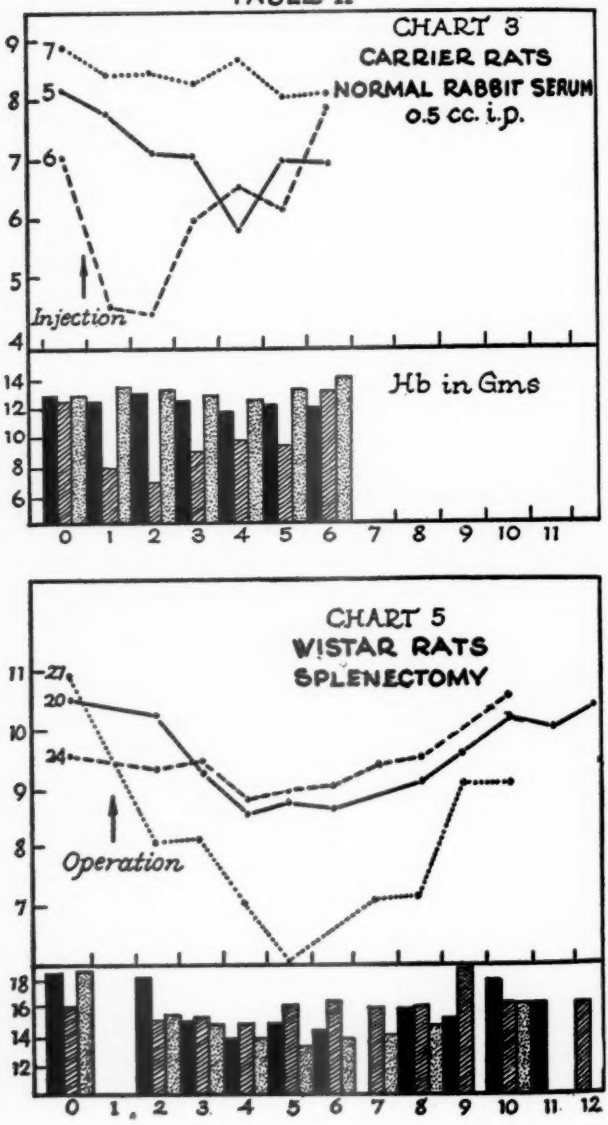


CHART 3
CARRIER RATS
NORMAL RABBIT SERUM
0.5 cc. i.p.
Injection
Hb in Gms
CHART 5
WISTAR RATS
SPLENECTOMY
Operation

RES does play some part in the defense of the animal." It could, therefore, be expected that an antiserum directed against the multiform cellular antigenic elements of the spleen, including those of the reticulo-endothelium, would be a potent and biologically specific blockading factor. If this is correct, then a partial or complete depression of the splenic function should follow this injury with manifestation of acute bartonellosis and anemia in the carrier rat.

The first group of 4 adult carrier rats was treated with the anti-rat spleen rabbit immune serum (REIS). Each rat was injected intraabdominally with 0.5 cc. of the undiluted serum (Complement fixation titer 1:1600). A sharp decline was noted within the following twenty-four hours in all 4 rats. This process was progressive, reaching within the next forty-eight hours values as low as 4 and 3 million. Simultaneously, there was a sharp decline of the hemoglobin content (Table I, Chart 2). The lowest erythrocyte counts of 2,920,000 and 2,070,000 were recorded in rats Nos. 4 and 16 on the fifth day after serum injection, with the appearance of bartonellae in rat No. 16 before its death. During the subsequent period of observation, intraerythrocytic and extracellular bartonellae appeared in rats Nos. 4, 9 and 10 as minute elongated diplococci or as delicate rods, singly and in groups and mostly in the polychromatophilic cells. Beginning with the tenth day of observation the bartonellae have disappeared, the hemoglobin content rose to 13 Gm and both rats recovered.

The next series of 7 carrier rats was similarly treated by a single i.p. injection of 0.5 cc. of the same homologous REIS. Blood counts were made five hours after the administration when in 2 of them a rapid decline in red cells was noted. Within the following twenty-four hours the values for red cells had fallen in both rats to 3 millions and the hemoglobin content to 7 Gm with the appearance of bartonellae on the fifth day. One of these rats died on the seventh day after serum injection. The remaining rats of this series also responded with anemia; however, the reactions were less severe, with the lowest values of 4 million red cells and hemoglobin content of 6.8 Gm (Rat

No. 12). This rat also developed a visible bartonellosis at the eighth day after injection. A longer observation (seventeen days) has shown that similarly to splenectomy the injection of REIS may induce late reactions and relapses. This was observed in some rats injected with 1.0 cc. REIS i.p. In one of them an initial drop to 5 million red cells (over 9 million before injection) and a hemoglobin content to 6.6 Gm (14.5 Gm before injection) was observed seventy-two hours after injection, falling to 2,830,000 RBC and 5.6 Hb on the fourteenth day of observation (Rat No. 35). Another rat of this series (No. 34) responded with values of 2,400,000 RBC (10 million before injection) and hemoglobin content as low as 5.4 Gm also at the end of seventy-two hours after serum injection. A gradual increase in blood values was then observed daily, reaching 6,610,000 RBC and 11.8 Gm Hb on the tenth day, after which time a decrease ending with 2,530,000 RBC and 4.1 Gm Hb and bartonellosis occurred on the fifteenth day. In other words, an attack of acute anemia resulted from a single injection of REIS and was followed by a relapse accompanied by a visible bartonellosis after eleven days.

As compared with the prompt reaction of rats injected with 1.0 cc. of REIS the response to 0.5 cc. of serum was slower and the blood changes milder. In fact, none of the rats of this series (Nos. 36, 37, 38) developed a severe anemia or bartonellosis. Blood values of 4,790,000 (Hb 9.4 Gm) were the lowest recorded on the seventh day after injection in rat No. 38 while in the 2 others the range was between 6 and 8 million red cells and 10 to 15 Gm Hb. In analyzing these results it has to be considered that the reaction depends not so much on the amount of the injected serum as on its potency evaluated by the complement fixation titer. The acute anemia and bartonellosis in rats Nos. 4, 9, 10 and 16 (Table I, Chart 2) were induced by REIS of an exceptionally high titer (1:1600). The titer of REIS used in the later series of rats could not be established due to the anticomplementary action of the antigen.

As can be judged by the qualitative changes the blood picture corresponds to that of splenectomized carrier rats. The most prominent cellular changes, namely, the appearance of

macrocytes, normoblasts, poikilocytosis and polychromatophilia occurred in rats treated with REIS of high titer (1:1600) and which developed bartonellosis and anemia. The blood examination of other rats in which only transient and moderately low blood values were found did not reveal any cellular abnormalities except polychromatophilia and anizocytosis.

Similarly to splenectomized carrier rats there was a definite leucocytosis in rats injected with REIS. Some reacted with a leucocytosis before any signs of anemia were observed. Usually an increase in white cell count was noted within 48 hours after injection, the average figures being between 25 and 45 thousand with a maximum of 69,300 recorded 72 hours after injection of 1.0 cc. of REIS (Rat 31). The splenectomized partner (Rat 30) developed two days later 76,600 white cells. The differential white cell count offered also a very similar picture, namely, a neutrophilia (up to 82%) at the expense of lymphocytes.

C. Control Experiments

These experiments were of particular importance since it has been observed that various blockading chemical agents or associated infections, intoxications, etc. may produce a transitory or occasionally a severe bartonellosis and anemia in carrier rats (Weinman, 1944, p. 306).

This series was carried out in the following lines: 1) carrier rats treated with normal serum; 2) Wistar rats treated with homologous REIS; 3) carrier rats treated with heterologous REIS.

Of six carrier rats injected with fresh serum from a normal rabbit (0.5 cc. i.p.) only one (No. 6) reacted with a drop in red cells and hemoglobin 5 hours after injection (Table II, Chart 3). This immediate depression was, however, of transient character, reaching rapidly the normal level. The remaining 5 rats showed fluctuations of the red cell counts between 7 and 9 million with only slight changes of hemoglobin content. No cellular changes in blood films were observed.

As can be seen from Charts 5 and 6 (Tables II and III) the response of Wistar rats to the homologous REIS (titer 1:1600)

is comparable to that of splenectomy. With the exception of a temporary decline on the fifth day after REIS injection or splenectomy (Rats 22 and 27 respectively) the blood values remained within the normal limits throughout the period of observation. The same applies to hemoglobin and to the number of white cells.

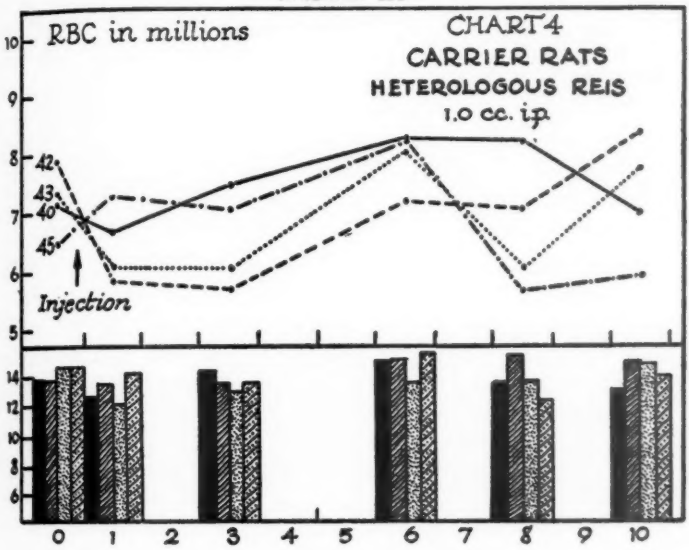
Finally, attempts were made to influence the latent bartonella infection in carrier rats by heterologous REIS, i.e. by serum of rabbits immunized with spleen and bone marrow of guinea pigs or chicks. Three rats of the carrier stock were injected with 1.0 cc. each of anti-chick REIS and another 3 rats of the same stock with anti-guinea pig REIS. It can be seen from Chart 4 (Table III) that rats 42 and 43 (anti-guinea pig serum) after a moderate reaction of 3 days duration have regained normal blood values by the tenth day. Rat No. 40 (anti-chick serum) remained normal while rat No. 45 (anti-chick serum) showed a decline in red cells beginning with the sixth day. The hemoglobin values remained in all rats of this series unchanged. No response was noted in rats Nos. 41 and 44 not shown on the chart. In none of the rats were the white cells affected.

Summary and Conclusions

An attempt has been made to determine the biological properties of the reticulo-endothelial immune serum (REIS) *in vivo*, particularly its inhibitory action on the defense mechanism of the host. The albino nonsplenectomized bartonella carrier rat was chosen as an object to investigate the specific action of the REIS. This was done in view of the possibility of suppressing the physiologic function of the spleen which normally keeps the relationship between the host and parasite in equilibrium. In other words, an attempt was made to simulate the surgical removal of the spleen by a "biologic elimination" of the organ in attacking it by the specific anti-serum. The ultimate bartonellosis and anemia in rats treated with REIS served as manifestations of this action.

In the preparation of the specific anti-serum the technique of Bogomolets and Marchuk (1943) was followed in using

TABLE III


RBC in millions
CHART 4
CARRIER RATS
HETEROLOGOUS REIS
1.0 cc. i.p.
42
43
40
45
Injection

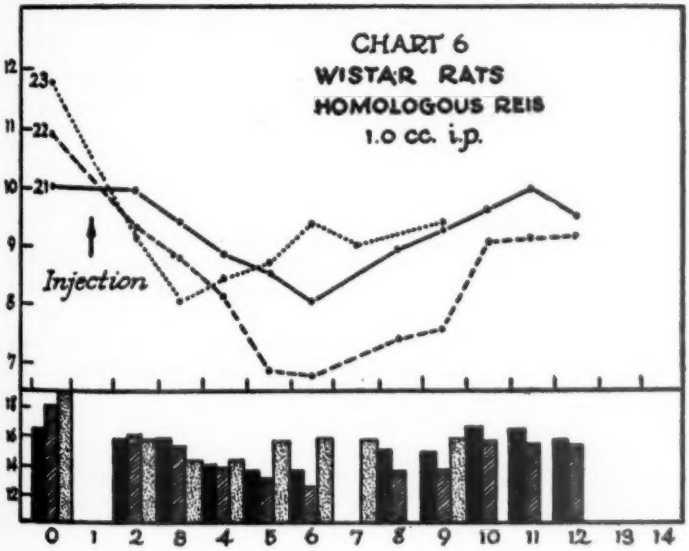


CHART 6
WISTAR RATS
HOMOLOGOUS REIS
1.0 cc. i.p.
23
22
21
Injection

spleen and bone marrow as antigens for immunization of rabbits. Homologous (anti-rat) and heterologous reticulo-endothelial immune sera (REIS) were used in various series of experiments.

The i.p. injection of 0.5 or 1.0 cc. of homologous REIS into 16 bartonella carrier rats produced a severe anemia in 11 rats, 6 of which developed an acute bartonellosis, with a fatal issue in 3 of them. The macrocytic, regenerative type of anemia corresponds to that produced in carrier rats by splenectomy.

No hematologic changes could be induced by the use of the same anti-rat REIS in the bartonella-free Wistar rats. For control experiments normal rabbit serum was injected into carrier rats without effect. Negative results were also obtained in using anti-chick and anti-guinea pig rabbit immune serum. It is believed, therefore, that the bartonellosis and anemia produced in some of the rats are due to a specific inhibitory action of the REIS on the defense mechanism of the host.

Legend to the Tables

Numbers on the curves represent individual rats. Solid line of the curves corresponds to solid blocks. Interrupted line corresponds to diagonal lined blocks. Stippled line corresponds to stippled blocks. No hemoglobin values are shown for Rat 16 (double line).

REFERENCES

The monograph of David Weinman (1944) which contains the most complete list of references on the various aspects of bartonellosis has been consulted frequently in this study. Only a few recent publications are listed below.

Anigstein, L., and Pomerat, C. M. Blood changes in the albino rat following administration of reticulo-endothelial immune serum (REIS). Fed. Proc. 4:111, 1945.

Bogomolets, A. A. Antireticular cytotoxic serum as a means of pathogenic therapy. Amer. Rev. of Soviet Med. 1:101–112, 1943.

Kessler, W. R. Studies on experimental Bartonella muris anemia in the albino rat. I & II. Jour. Inf. Dis. 73:65–84, 1943.

Marchuk, P. D. A method of preparing and preserving antireticular cytotoxic serum. Amer. Rev. Soviet Med. 1:113–123, 1943.

Pomerat, C. M. and Anigstein, L. Anti-reticular immune serum: its action demonstrated by tissue culture technique. Science, 100:456, 1944.

———— and Anigstein, L. Reticulo-endothelial immune serum (REIS): I. Its action on spleen *in vitro*. Tex. Rep. Biol. and Med. 3:122–141, 1945.

Weinman, David. Infectious anemias due to Bartonella and related red cell parasites. Trans. Amer. Philos. Soc. Vol. 33, Part 3, 1944, pp. 243–350.

HOOKWORM DISEASE IN TEXAS

by

J. ALLEN SCOTT*

In the early history of hookworm disease in the United States, Texas played an important part. The credit for recognizing the widespread and serious nature of the infection belongs, of course, to Dr. Charles Wardell Stiles. For Texans it is interesting that the first printed statement of his conclusions to this effect was published in the Texas Medical News (Stiles 1901). Apparently Dr. Allen J. Smith, then a professor in the Medical School of The University of Texas, was the first person to see and recognize hookworm eggs in the stools of a native of the United States. Although these findings were not published until 1901 after several other people had published similar observations, he did see them in 1895 and demonstrated them at that time to his students. The facts were first reported by Schaefer (1901)[1] who also mentioned the finding of ova in the feces of two out of eight students examined in the Medical School at Galveston. These studies were continued and Smith (1903) reported that the ova had by then been demonstrated in the stools of eight out of eighty-six students who had always lived in Texas. Of this finding Stiles (1903) remarks "It meant a practical demonstration that uncinariasis was more or less widespread; and since light cases occurred among medical students, heavier infections must naturally occur among persons who come more regularly in contact with the dirt."

DISTRIBUTION AND PREVALENCE OF HOOKWORM DISEASE IN TEXAS

Very early in the study of hookworm disease Stiles (1903) made a series of general observations with regard to the dis-

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[1]Smith (1903) notes that Schaefer incorrectly gave the date as 1893.

tribution of the disease in the southern United States, among which the following have stood the test of time:

1. "Infections occur chiefly in rural districts."
2. "Infection occurs chiefly on sandy soil."
3. "Severe cases are more common in women and children than in men over twenty-five years of age." (Under the changed conditions today this statement should perhaps now be modified to read, "More common in children than adults.")
4. "Several cases are likely to occur in the same family."
5. "Whites appear to be more severely affected than negroes."

These conclusions of Stiles led Smith (1903) to publish a map showing the distribution of the homes of the affected students and to point out that they all came from the sandy regions of East Texas. Thus it was clear at that early date, that hookworm disease in Texas was confined to the eastern part of the state, and one important reason for this localization, the sandy soil, was already recognized. We now know that another reason for the localization is the distribution of rainfall. Observations in other parts of the world have shown that hookworm disease is prevalent only where the annual rainfall is above 40 inches, a condition which is fulfilled in Texas only in the eastern part.

Subsequent to 1903 an increasing number of observations were made on the prevalence of hookworm disease in Texas, but the first thorough surveys were started in 1912. The best data on which to base conclusions with regard to the prevalence of hookworm disease in Texas during these early years are those furnished by the reports of the Rockefeller Sanitary Commission for the Eradication of Hookworm for the years 1912 to 1914.[2] In figure 1, the prevalence percentages obtained from these studies are shown by counties on a map of the region surveyed. With regard to the selection of persons to be examined these reports stated: "This survey is based on a microscopic examination of at least two hundred children between the ages of six

[2]Since this work was carried out in coöperation with the State Board of Health, the same data are reported by Boerner (1915).

TABLE I

The prevalence of hookworm infection in school children of Texas in 1912 to 1914 as compared with 1933 to 1944.

County	1912–1914 Source*	Number examined	Per cent Positive	1933–1944 Source*	Number examined	Per cent Positive
Anderson	3	1,047	35	4	40	15
Angelina	1	1,096	75			
Austin	2	372	6			
Brazoria	2	305	10			
Brazos	2	677	5			
Burleson	3	380	12			
Cass	3	1,351	85			
Colorado	3	221	<1			
Falls	3	492	0			
Fort Bend	3	1,018	<1			
Freestone	3	1,074	47			
Galveston	3	1,898	<1			
Grimes	2	804	48			
Hardin	1	1,114	61	6	108	34
Harris	2	1,665	56			
Henderson	2	950	27			
Houston	2	706	55			
Hunt	3	1,008	3			
Jasper	1	1,016	59	6	957	47
Jefferson	2	526	12			
Kaufman	3	608	19			
Liberty	2	600	60			
Milam	3	376	10			
Montgomery	1	999	71			
Nacogdoches	3	1,034	49	4	126	24
Navarro	2	1,169	14			
Newton	..	....	..	6	749	52
Orange	2	407	47	6	717	8
Panola	2	517	38	4	203	28
Polk	2	861	57			
Red River	3	286	20			
Robertson	2	275	21			
Sabine	3	496	71	4	312	38
San Augustine	2	534	84	4	370	45
San Augustine	..	....	..	6	205	33
San Jacinto	2	626	87			
Shelby	2	1,151	37			
Smith	2	525	11	5	216	20
Upshur	3	250	63			
Walker	2	839	57			
Waller	2	467	48			
Washington	3	624	2			
Wharton	3	1,806	2			

*Source of data:

1. Third Annual Report, Rockefeller Sanitary Commission for the Eradication of Hookworm Disease, for the year 1913.
2. Fourth Annual Report, Ditto, for the year 1914.
3. Fifth Annual Report, Ditto, for the year 1915.
4. Upton, R. G., 1936.
5. Kelly, L. F., A. Statistical Report on Intestinal Parasites as found in Smith County. Texas Society of Medical Technologists, 1943.
6. "Statistical information on the incidence of hookworm in six East Texas Counties, June, 1942–May, 1944." Furnished by Division of Parasitology, Bureau of Laboratories, State Department of Health.

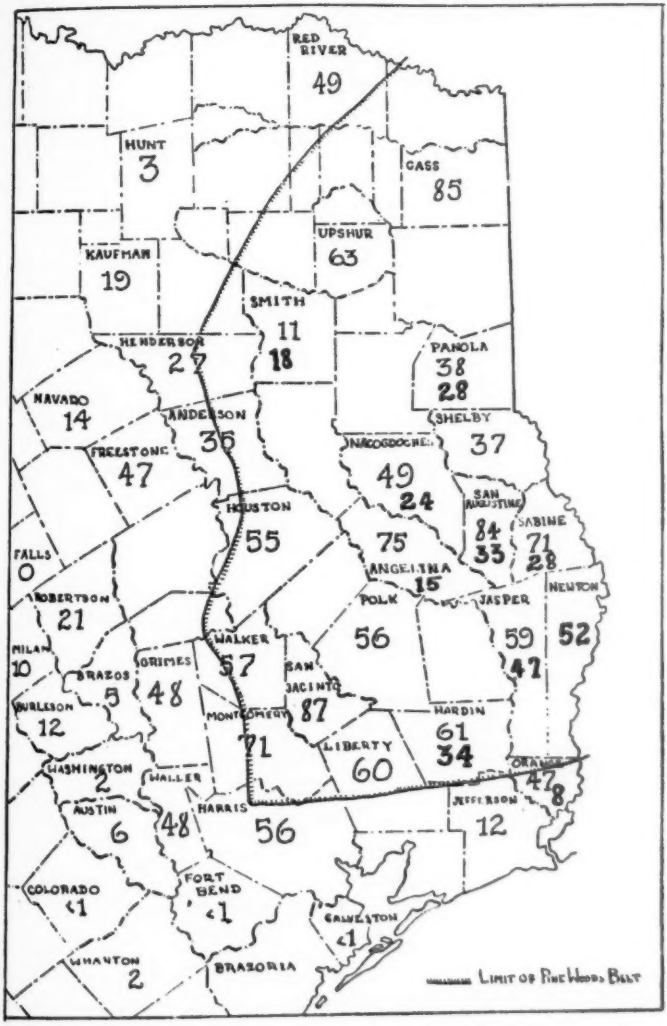


Figure I.—Map of east Texas showing the prevalence percentage of hookworm by counties for the years 1912–1914 (upper figures) and for the years 1933–1944 (lower figures). Data from Table I.

and eighteen, taken at random from rural districts distributed over the country."

On this map there is also shown the limit of the pine woods belt of east Texas, a belt which is practically coincident with the sandy soil region of east Texas. West of this belt the rainfall is less than 45 inches per year whereas within the belt the rainfall increases to over 50 inches at the Louisiana border. It is obvious from this map that most of the hookworm infection of that time was in this pine belt.

Today the distribution of infection seems to be further localized. Table I summarizes the early studies already mentioned and shows comparative figures for recent dates.

Prevalence data from recent examinations have also been placed on the map (Figure 1), but they are not strictly comparable with the early ones, since modern methods of examination are more efficient and tend to produce higher percentages. Moreover, the more recent studies did not use exactly the same type of random sampling, i.e. since the infection is not evenly spread throughout most of the counties, considerable divergence of results might be produced by the way the children were chosen for inclusion in the study.

In spite of these differences, it seems safe to draw two tentative conclusions. First, it may be concluded that in the counties outside of the pine woods belt the prevalence has dropped to a very low figure. For example, in Orange County, there has been an apparent drop from 47 to 8 per cent. Even though these two figures are not strictly comparable, information obtained from private physicians and local health departments indicate that nowhere outside of the pine woods area is the prevalence now very high. Second, it is to be concluded that in the heart of the pine woods belt where the soil is extremely sandy (in other words from about the center of Nacogdoches County east to the Louisiana border and south to the southern borders of Jasper and Newton counties) the prevalence seems to have dropped to about half of what it originally was. In other words, roughly one half as many people are infected with hookworm in these counties as were infected in the days before any control work had been done.

Clinical Hookworm Disease in Texas

Important as it is to know what proportion of the people of any region are infected with hookworms, it is still more important to know how many are showing clinical symptoms of the disease. Today all of the authorities specializing in the study of hookworm disease agree that in most parts of the world relatively few hookworm infections produce any symptoms. By and large this is a quantitative matter, i.e. most infected people harbor only a few worms and are able to completely compensate for whatever damage may be done. Those who harbor larger numbers of worms, however, may reach the point where they are no longer able to compensate sufficiently and symptoms of anemia appear. Some people can compensate for the effects of a larger number of worms than others depending on the nutritional status and other factors, but on the average there is a definite correlation between the degree of anemia and the number of worms harbored.

This quantitative viewpoint is considered by some to be a very modern one and quite radical. That such is not the case is witnessed by the following quotation from Boerner (1913) who was then the Texas State Director of the Hookworm Commission: "Not all of these were sufferers of the disease; however, they were infected, were carriers, and as such were capable of spreading the infection. The most heavily and most frequently infected class was found to be the school children." Workers at that time were not equipped with methods which would permit either adequate assessment of the size of the infection or the amount of damage done, and it was therefore impossible to implement the idea.

The necessary methods were not long in being developed. As early as 1915 Darling, Barber, and Hacker (1920) working in Malaya introduced the worm count method, i.e. stools passed after thorough anthelmintic treatment were washed and screened making it possible to recover and count the worms removed by the drugs. With this information as to the number of worms harbored, supplemented by the results of hemoglobin determinations, these authors were able to show that the degree

of anemia was correlated with the number of hookworms harbored by the individual. In other words, under the living conditions existing there, the people with few hookworms were no more anemic than those harboring none, while on the average those with the largest number of worms had the most profound anemia. These conclusions were confirmed and emphasized again and again by various investigators, including Darling and other members of the Rockefeller Foundation staff which was then engaged in hookworm campaigns throughout the tropical world.

In 1922 Cort and a group of colleagues began a series of studies to define and amplify this quantitative aspect of the disease. Early in their work a dilution egg count method was developed by Stoll (1923) by which an estimate of the number of worms harbored by the people could be obtained relatively quickly and without the laborious and disagreeable task of worm counting. Other groups of workers adopted this method and helped develop the quantitative idea, so that by 1925 the principal organizations engaged in hookworm control throughout the world had completely adopted the quantitative approach in their work.

As far as can be determined, the only work in Texas which has made use of these quantitative methods is that of Upton (1933, 1936). His conclusions were based on the examination by egg counting methods of nearly 2,000 school children from several counties in the known hookworm district of east Texas. He found that the prevalence of infection varied in different rural schools from 7 per cent to 84 per cent. More significant, however, is his statement that of all the positive cases only one per cent could be classed as having the "heavy infections" which are generally interpreted as almost always producing symptoms.

Before attempting to draw conclusions as to the significance of these data one should consider the factors which are now recognized as determining whether hookworm infections will be clinical or subclinical. These factors can be summarized as follows:

1. The damage done by hookworms seems to be caused solely by their blood sucking activities.

2. On the average the more worms present in the intestine, the more blood will be lost.

3. Since hookworms do not multiply in the body, the number present is governed by the number acquired and by the resistance of the body.

4. Therefore, the amount of damage caused by the infection depends upon: (a) opportunity to acquire infection; (b) ability to resist the infection and its effects.

5. The opportunity to acquire infection is limited by the climate and the nature of the soil to the eastern part of Texas; is limited by sanitary conditions now existing in the state to rural districts, and within these districts almost exclusively to people living under the poorest economic circumstances.

6. The ability to resist the infection and its effects depends primarily on the nutritional status of the individual.

7. Persons on adequate diets apparently acquire an immunity from their first infection which enables them to resist the establishment of large numbers of worms.

8. Persons on adequate diets are also better able to compensate for blood loss than are the malnourished.

9. The primary dietary deficiencies responsible for the lack of immune response and for the inability to adequately compensate for blood loss seem to be iron and other minerals, animal proteins, and vitamin A.

10. The occurrence of serious hookworm disease, as contrasted to the subclinical state, seems therefore to depend upon poor sanitary conditions in regions of high rainfall and sandy soil; and on the presence of a certain degree of malnutrition. Both of these causes are intimately related to economic conditions.

With these factors in mind we can now attempt to marshal the available data bearing on the present status of hookworm disease in Texas. The data presented show that outside of a small area south and east of Nacogdoches and north of the Orange County line the prevalence is very low and very few cases of hookworm disease are now encountered by the

physicians. Within this area not more than 50 per cent of the children of any county are infected. In some local areas the percentage may be much higher than this, but the average over the entire rural area of the county will usually be much lower.

In general the severity of the symptoms are correlated with the prevalence of infection. In other words in those parts of a county where a high percentage of the children are infected, a large number of clinical cases are to be expected. Where the prevalence is low, the clinical cases are generally few. In Alabama, Smillie and Augustine (1926) concluded that children with "heavy" infections, i.e. those passing more than 11,100 eggs per cc of stool, practically always showed clinical symptoms. Upton (loc. cit.) found only one per cent of the infected children of east Texas to fall in this group. In a second group called "moderate," i.e. between egg count limits of 2,100 and 11,099 per cc, only a part of the children can be expected to show symptoms. Upton found 17 per cent of the infected children to be in this group. The remaining 82 per cent were passing fewer than 2,099 eggs per cc and according to the Alabama studies would seldom be found to present any symptoms.

Upton's data have also been compared with standards in use in Georgia (Andrews 1942) by plotting them on a logarithmic scale (Scott 1942, 1946). The results of this comparison showed that 95 per cent of the infected Texas children fell into a group having infections of such a small size as to never cause symptoms under the living conditions of Georgia.

It must be recognized, of course, that there is some danger of carrying such conclusions from state to state. After comparing the living conditions in east Texas with those in the other southern states and gathering information from physicians of the area, I believe it is safe to hazard a guess that not over 10 per cent of the infected children show any clinical symptoms. In the above mentioned area east and south of Nacogdoches, there are approximately 25,000 white children between the ages of 1 and 15 living under rural conditions. Assuming 50 per cent to be infected and 10 per cent of these to show clinical symptoms we reach a figure of 1,250 cases of hookworm

disease. It should be noted that all of these figures are the estimated maximum figures and the true number is likely to be somewhat less than 1,000. That we have had to arrive at this figure by a process of controlled guessing should emphasize the need for further scientific studies to determine accurately the importance of hookworm disease in the public health picture of east Texas. In a later paper suggestions as to appropriate measures will be presented.

Summary and Conclusions

The available data indicate that in most of the parts of Texas where hookworm was once common, the infection now occurs only in a very small percentage of the population and clinical cases are a rarity. In a small area in the extreme eastern part of the state the infection is more common, but even there it is not more than half as frequent as it once was. A rough estimate based on the meager information available indicates that probably not more than 10,000 persons are infected and the number of clinical cases probably does not exceed 1,000. The improvement over conditions known to exist 30 years ago has probably been due in part to the control programs of various sorts. On the other hand, in spite of depression set backs, there has been a general lifting of the economic level of living in these areas which has undoubtedly been reflected in the decrease of hookworm prevalence through improved nutritional and sanitary conditions. There is a great need for further investigations to determine the exact public health status of hookworm disease at the present time and to define the conditions which might be modified so as to effect further control.

BIBLIOGRAPHY

Andrews, J.: Modern views on the treatment and prevention of hookworm disease. Annals Int. Med. 17:891–901, 1942.

Boerner, M. H.: The hookworm problem; Synopsis of the work of the Hookworm Commission of the Texas State Board of Health. Texas State Jour. Med. 9:133–34, 1913.

Boerner, M. H.: Presence, prevalence, and geographic distribution of hookworm infection in Texas. Texas State Jour. Med. 10:377–78, 1915.

Darling, S. T., Barber, M. A., and Hacker, H. P.: Hookworm and malaria research in Malaya, Java, and the Fiji Islands, 1915–1917. Internatl. Health Bd. Pub. No. 9. Rockefeller Foundation, N.Y., 1920.

Schaefer, M. C.: *Anchylostoma duodenale* in Texas. Med. News, 79:655–658, 1901.

Scott, J. A.: Simplified quantitative methods for hookworm control programs. Amer. Jour. Trop. Med. (in press), 1946.

Scott, J. A.: The natural pattern of dilution counts of helminth eggs. Amer. Jour. Trop. Med., 22:647–654, 1942.

Smillie, W. G. and Augustine, D. L.: Hookworm infestation. The effect of varying intensities on the physical condition of school children. Amer. Jour. Dis. Children, 31:151–168, 1926.

Smith, A. J.: Uncinariasis in Texas. Amer. Jour. Med. Sci. 126:768–798, 1903.

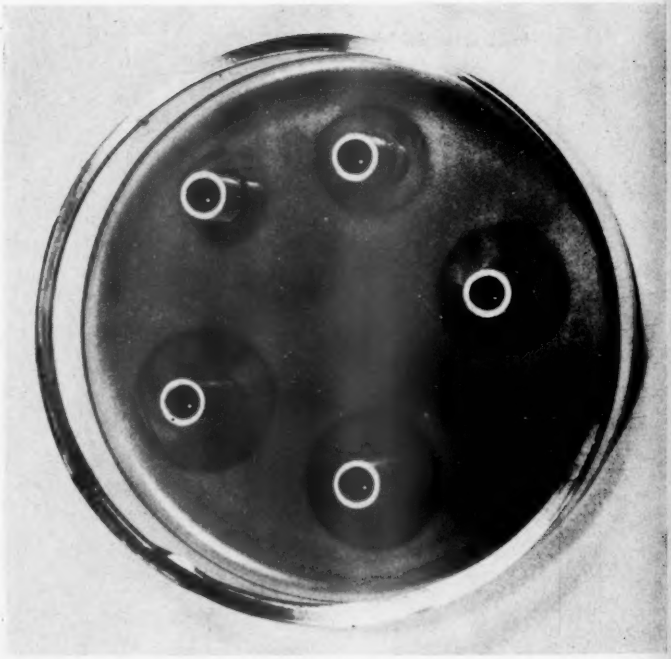
Stiles, C. W.: Uncinariasis (anchylostomiasis) in man and animals in the United States. Texas Med. News, 10:523–532, 1901.

Stiles, C. W.: Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States; Hygienic Lab. Bull. no. 10, 1903.

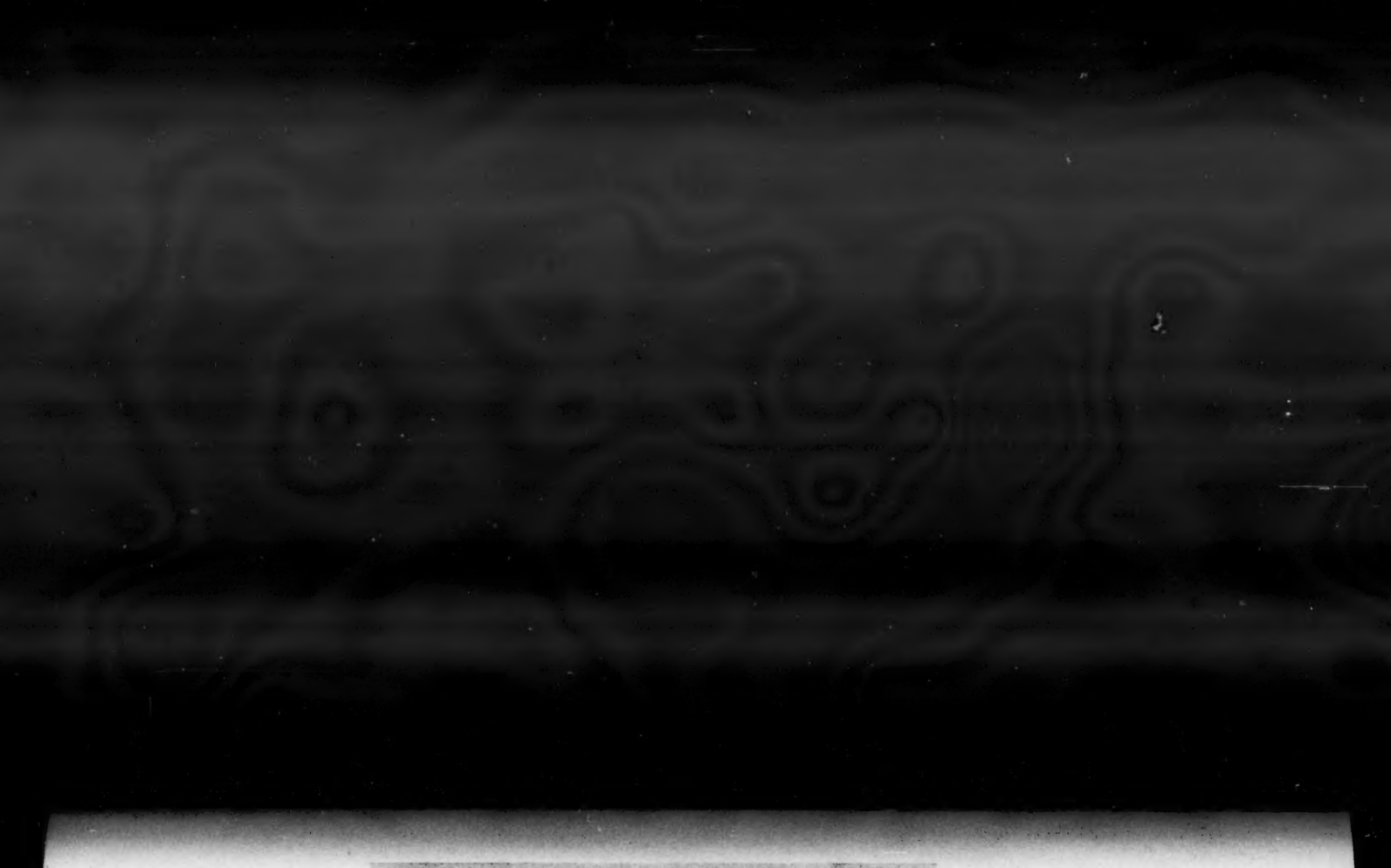
Stoll, N. R.: Investigations on the control of hookworm disease. XV. An effective method for counting hookworm eggs in feces. Amer. Jour. Hyg., 3:59–70, 1923.

Upton, R. G.: Hookworm, the forgotten problem; East Texas Mimeograph Bull., Texas State Board of Health, Jan. 1933.

Upton, R. G.: Incidence and severity of hookworm infestation in east Texas. Amer. Jour. Pub. Health and The Nation's Health, 26:924–926, 1936.



Penicillin Inhibition of *Staphylococcus aureus*
(Photograph reproduced through the courtesy of Eli Lilly and Co.)



THE NATURE AND ACTION OF THE ANTIBIOTICS

EDWARD H. FRIEDEN*

This review represents an attempt to correlate the most important available data relating to the nature, biological activity, and mode of action of the antibiotics. The wider field of therapeutic application has not been treated, inasmuch as this phase of the subject has received extensive consideration elsewhere. While no claim is made for an exhaustive survey of the literature, it is believed that the discussion includes essentially all of the specific chemical entities, however poorly defined, which have been shown to exhibit antibiotic activity.

For the purposes of this review, the term antibiotic may be defined as follows: a chemical substance, produced by one or more living organisms, which possesses more or less selective antimicrobial properties at relatively low concentrations. These properties may be either inhibitory or lethal. While some antibiotics may exhibit lytic properties, lysis is in general unrelated to antibiotic activity.

Although definite knowledge of antibiotics as chemical entities is of relatively recent origin, the existence of such substances has been suspected almost from the dawn of bacteriological science. As early as 1877 Pasteur demonstrated that in the presence of other bacterial species, the development of the anthrax bacillus is repressed. Studies on the role of the soil in the etiology of disease led to the discovery that the disappearance of certain pathogens from the soil is extremely rapid, and the suggestion that this disappearance is due to the antagonistic effects of other microorganisms is to be found very early in the literature of bacteriology.

The first studies upon antagonistic relationships among bacteria were complicated by the fact that the multiple nature of such antagonisms was not always recognized. It seems likely that some of the earliest reports of growth inhibition of one

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species in cultures of another were actually due to effects such as simple exhaustion of the medium, change in pH, or space antagonism. However, Emmerich and Löw (1901) definitely established the presence of lytic (pyocyanase) fractions in cultures of *Ps. aeruginosa (Ps. pyocyanea)*, and suggested the local use of this substance in the treatment of certain infections. Other evidence for the elaboration of toxic factors by *B. subtilis*, *B. mesentericus*, and *B. mycoides* was presented by Nicolle (1907), Rosenthal (1925) and Much et al. (1924, 1925). Probably the effects observed by these workers were not entirely due to antibiotic production; Hettche and Weber later (1939) showed that *B. mesentericus*, for example, produces isovaleric and oleic acids in concentrations high enough to be bactericidal for some organisms, and that the ether-extracted filtrates were virtually inactive.

Wrede and Strack (1924) demonstrated the presence of the bactericidal substance pyocyanine in cultures of *Ps. aeruginosa*. The extreme toxicity of this material prevented its clinical application.

Gratia and Dath in 1924 described an antibiotic metabolic product of certain strains of actinomycetes, to which the designation 'actinomycetin' was applied. This substance was shown to have a wide range of antibacterial activity, but the subject was not pursued further. It is likely that 'actinomycetin' is similar, if not identical with the antibiotics later studied by Welsch (1941) and by Waksman and co-workers (1941, 1942).

Only scattered references to the elaboration of antibiotics by various bacteria appeared in the literature during the next fifteen years. It remained for the important discoveries of Dubos (1939) to stimulate more widespread interest in bacterial production of antimicrobial agents. Dubos treated samples of various soils with suspensions of pathogenic bacteria, and was then able to isolate a spore-forming bacillus which produced the potent antibiotic now known as tyrothricin. Subsequently, the production of antibiotics by bacterial cultures was reported by Hoogerheide (1940), Waksman, et al. (1941, 1942), Gardner and Chain (1942), Jansen and Hirschman (1944), Oxford (1944), and Guase and Brazhnikova (1944).

The first instance of antibiotic production by fungi was apparently the isolation of penicillic acid by Alsberg and Black in 1913. These authors found that this substance, a metabolic product of *Penicillium puberulum*, was effective against a wide variety of microorganisms.

In 1929, the classic studies of Fleming upon penicillin, a metabolite of *Penicillium notatum* appeared. In this pioneer work Fleming studied the effect of penicillin upon a variety of bacterial species, and demonstrated the refractoriness of most gram-negative organisms. He also showed the usefulness of penicillin in preventing contamination of cultures of gram-negative forms, and demonstrated the relative non-toxicity of penicillin to animals and leukocytes.

Although a few reports dealing with the activity of penicillin solutions appeared between 1929 and 1940, it was not until the latter date that the extensive investigations of the Oxford group dealing with the preparation and clinical application of potent penicillin concentrates were reported. Speculation concerning the reason for this prolonged period of relative inactivity is of some interest. Doubtless the difficulty of obtaining fractions of even moderate activity was an important factor in the delay. It is also possible that the concomitant rise of the sulfonamides as a popular chemotherapeutic agent focused the attention of many investigators elsewhere.

Penicillin continues to occupy the topmost position in the list of important and useful antibiotics. The list of antibiotic substances which are produced by molds and fungi is, however, a large one, and is still expanding. Historically, the reports of Weindling and Emerson (1936), Waksman, Horning and Spencer (1942), and of the group of investigators headed by Raistrick are significant. The latter had been interested in the chemical composition of the metabolic products of molds for some time prior to 1940. The early reports of antibiotic production by similar organisms stimulated Raistrick to a reinvestigation of some of the compounds which had been isolated previously, and a number of them were found to possess antimicrobial properties.

Recent systematic investigations of molds and fungi have revealed that the elaboration of antibiotic substances is a characteristic property of large groups of these organisms.

While most discussions of antibiotic agents do not include the various bacteriophages in this category, it is apparent that, despite some uncertainty as to its true nature, bacteriophage fulfills the essential conditions required for classification as an antibiotic. The distinct differences in both the nature and the mode of action of bacteriophage from the rest of the members of this group are such as to make it undesirable to include it in the present discussion. Comprehensive summaries dealing with the nature and activity of bacteriophage include those of d'Herrelle (1930), Krueger (1936), Krueger and Scribner (1941), and Delbruck (1942).

Antibiotic activity is not limited to products of bacteria and fungi, but has been observed in cultures of algae (Pratt, et al., 1944), in higher plants (Osborn, 1943), and the antibiotic activity of at least one animal product, lysozyme, is well established (Fleming, 1922).

Nature and Source of Antibiotics

Any rapidly expanding field of scientific endeavor is attended almost inevitably by a certain amount of confusion. This confusion is exaggerated in the study of antibiotics for a variety of reasons. The past five years have witnessed an extremely rapid development in this field, perhaps more rapid than any hitherto observed. Bacteriological and mycological taxonomy is by no means completely clarified; this fact has led, in many instances, to some uncertainty as to the exact nature of the organism being studied. Recently it has become evident that many fungi produce more than distinct substance with antibiotic activity, and it has also been shown that more than one fungal species may produce substances indistinguishable by either chemical or biological means. Finally, the fact that few antibiotics have as yet been obtained in a purified state has prevented precise comparison of the products isolated by different investigators.

Up to May, 1945, the literature records some thirty different substances possessing antibiotic activity which are quite definitely distinct compounds. These are listed in table I, together with their sources, the general chemical nature where this is known, and the approximate range of antibacterial activity.

The antibacterial activity of the products listed above will be considered later in more detail, the effectiveness against gram-positive and gram-negative forms being a useful, if rather superficial, means of classification. It should be noted that this classification is a relative one only; many of the antibiotics which are generally considered specific for gram-positive forms are also bacteriostatic for gram-negative organisms in sufficiently high concentrations.

In table I the list of organisms from which the various antibiotics have been isolated is abbreviated, the sources given being historically or economically important. There is little doubt that the same antibiotic can be produced by more than one organism, although the organisms responsible are generally rather closely related taxonomically. As has already been noted, this fact has been responsible for some confusion in the literature. The case of clavacin is an excellent example.

In 1942, Waksman, Horning and Spencer isolated an impure metabolite of *Aspergillus clacatus* possessing antibiotic activity, which they named clavacin. Almost simultaneously, Wiesner (1942) published experimental results nearly identical with those of the Waksman group. In the same year, Chain, Florey, Jennings, and Callow described the isolation of crystalline claviformin, an antibiotic metabolite of *Penicillium claviforme.* In 1943, Raistrick, et al., reported the results of an extensive chemical, pharmacological, and clinical investigation of patulin, obtained from *Penicillium patulum.* Somewhat later a crystalline substance was isolated from *Aspergillus clavatus* by Bergel and his associates; the compound was named clavatin and was subsequently shown to be identical with claviformin and with patulin. Florey, Jennings, and Philpot (1944) demonstrated that claviformin appeared as a metabolite of *Aspergillus giganteus* Wehm under the proper cultural conditions, and Karow and Foster (1944) reported the isolation of clavacin

TABLE I

A Summary of the Important Characteristics of the Antibiotics

Compound	Produced by	Chemical Nature	Range of Activity
I. ***Substances Produced by Bacteria***			
1. Actinomycin	*Actinomyces antibioticus*	N-containing quinone	Gram-positive bacteria and fungi
2. Diplococcin	*Streptococcus sp.*	Low mol. wt. protein or polypeptide	Gram-positive cocci
3. Gramicidin	*Bacillus brevis*	Cyclic polypeptide	Gram-positive organisms
4. Gramicidin S	*Bacillus sp.*	Polypeptide	Gram-positive and Gram-negative organisms
5. Iodinin	*Chromobacterium iodinium*	Phenazine derivative	Gram-positive cocci
6. Pyocyanase	*Pseudomonas aeruginosa*	Unsaturated fatty acid mixture	Gram-positive and Gram-negative organisms
7. Pyocyanine	*Pseudomonas aeruginosa*	Di-(α -oxyphenazine) derivative	Gram-positive organisms
8. Proactinomycin	*Proactinomyces sp.*	Organic base	Gram-positive organisms
9. Streptomycin	*Actinomyces griseus*	Organic base	Gram-positive and Gram-negative organisms
10. Streptothricin	*Actinomyces lavendulae*	Organic base	Gram-positive and Gram-negative organisms
11. Subtilin	*Bacillus subtilis*	Organic acid	Gram-positive organisms
12. Tyrocidine	*Bacillus brevis*	Cyclic polypeptide	Gram-positive and Gram-negative organisms
II. ***Substances Produced by Fungi***			
1. Aspergillic acid	*Aspergillus flavus*	γ-pyrazone derivative	Gram-positive and Gram-negative organisms
2. Chaetomin	*Chaetomium cochliodes*	No data	Gram-positive organisms
3. Citrinin	*Penicillium citrinum* *Aspergillus candidus*	Benzfuran derivative	Gram-positive and Gram-negative organisms
4. Clavacin	*Aspergillus clavatus* *Penicillium patulum* *Penicillium claviforme*	Substituted pyrone	Gram-positive and Gram-negative organisms

TABLE I—Continued

TABLE I—Continued

A Summary of the Important Characteristics of the Antibiotics

Compound	Produced by	Chemical Nature	Range of Activity
5. Fumigacin	*Aspergillus fumigatus*	High mol. wt. lactone	Gram-positive and Gram-negative organisms
6. Fumigatin	*Aspergillus fumigatus*	Benzoquinone derivative	Gram-positive organisms
7. Gliotoxin	*Gliocladium fimbriatum* *Trichoderma sp.*	Indole carboxylic acid peptide	Gram-positive organisms
8. Notatin	*Penicillium notatum*	Flavo-protein	Nearly all micro-organisms
9. Penicidin	*Penicillium sp.*	Organic base	Gram-positive and Gram-negative organisms
10. Penicillin	*Penicillium notatum* Other penicillia	Dibasic, N-containing acid	Gram-positive organisms
11. Penicillic acid	*Penicillium puberulum* *Penicillium cyclopium*	Unsaturated lactone	Gram-positive and Gram-negative organisms
12. Puberulic acid	*Penicillium puberulum*	Dibasic organic acid	Gram-positive and Gram-negative organisms
13. Puberulonic acid	*Penicillium puberulum*	Organic acid	Gram-positive and Gram-negative organisms
14. Spinulosin	*Penicillium spinulosin*	Benzoquinone derivative	Gram-positive and Gram-negative organisms
III. *Substances Produced by Algae and Higher Plants*			
1. Chlorellin	*Chlorella vulgaris* *Chlorella pyrenoidosa*	No data	Gram-positive and Gram-negative organisms
2. Crepin	*Crepis taraxacifolia*	α,β-unsaturated lactone	Gram-positive and Gram-negative organisms
3. Allicin	*Allium sativum*	Diallyl sulfone	Gram-positive and Gram-negative organisms
IV. *Substances Produced by Animals*			
1. Lysozyme	Many vertebrates	Protein	Many cocci

from a species of *Gymnoascus* and from an unidentified *Penicillium*. Independently of the work of Bergel, Chain, Florey, and Jennings (1944) established the identity of patulin and claviformin. Meanwhile, Waksman (1944) had obtained crystalline clavacin, and proved its identity with patulin, clavatin, and claviformin. It is therefore clear that the same antibiotic is elaborated by at least seven different fungal species.

A similar situation arose with respect to the antibiotics fumigacin and helvolic acid. Fumigacin was originally described (Waksman, Horning and Spencer, 1942) as a metabolic product of *Aspergillus fumigatus*. This material contained (Waksman, Horning and Spencer, 1943) 3.7 per cent nitrogen and melted at 185–186°. Later, Chain, Florey, Jennings, and Williams (1943) studied the antibacterial substance produced by *Aspergillus fumigatus*, mut. Helvola Yuill. This was observed to contain only carbon, hydrogen, and oxygen, and melted at 212°. Assuming it to be a substance not previously prepared, they applied to it the term helvolic acid. Later, however, Menzel, Wintersteiner, and Hoogerheide (1944) showed that Waksman's original fumigacin preparations could be separated into two distinct compounds—gliotoxin, which contained nitrogen and sulfur, and a nitrogen-free acid, m.p. 220°, which represented pure fumigacin. There exists no doubt that helvolic acid is identical with the latter substance.

The available evidence indicates that substances either identical with or closely related to penicillin are products of a large number of fungi. Philpot (1943) isolated an impure antibiotic from *Aspergillus giganteus* which he called gigantic acid. Its properties were suggestive of a very close similarity to penicillin. Flavacin, prepared by Bush and Goth (1943) from *Aspergillus flavus*, likewise appears to be closely related, and on the basis of solubility, stability, elemental composition and chemical behavior, and antibacterial activity, parasiticin, a metabolite of *Aspergillus parasiticus* (Cook and Lacey, 1944) can also be included in this category.

Foster and Karow (1945) examined a number of fungi for their ability to produce penicillin. They found that the solubility characteristics, stability (especially as a function of pH

and temperature), antibacterial "spectrum," and sensitivity to penicillinase of the antibiotics from *Aspergillus oryzae, Aspergillus nidulans, Aspergillus niger, Aspergillus flavipes,* and *penicillium citro-roseum* were indistinguishable from those of penicillin. Florey, Heatley, Jennings and Williams (1944) reported the formation of substances chemically and biologically similar to penicillin by five species of penicillia—*P. fluorescens, P. rubrens, P. avellaneum, P. baculatum,* and *P. turbatum.* Finally, the isolation of penicillin from *P. chrysogenum* has been reported by Smith (1942).

That the same species of fungus can produce more than one anti-bacterial substance was apparently first established by Coulthard, Michaelis, and Short (1941). Cultures of *Penicillium notatum* were found to contain an antibiotic different from penicillin; this was first named penicillin A but later referred to as notatin (Coulthard et al., 1942). The same antibiotic was independently isolated by Kocholaty (1942), who suggested the name penatin. Later Roberts et al. (1943) described "penicillin B," which had also been obtained from *P. notatum.* Subsequent studies made it clear that notatin, penatin, and penicillin B all represent the same protein by-product of *P. notatum* metabolism (Van Bruggen, et al., 1943; Birkinshaw and Raistrick, 1943; Kocholaty, 1943; Schales, 1943).

Waksman (1944) has pointed out that *Aspergillus fumigatus* apparently has the property of elaborating four different antibacterial compounds: spinulosin, fumigatin, fumigacin, and gliotoxin.

Less well defined antibacterials have been described by Penau, et al. (1943) (corylophillin from *Penicillium corylophilum*) and by Birkinshaw and others (1942) (from *Penicillium resticulosum*).

Systematic investigations of molds to determine the specificity of antibiotic production have been reported by at least two groups of workers. Wilkins and Harris (1942) summarized their studies on 100 fungal species, finding that activity (antibiotic) was characteristic of 40 per cent of aspergilli, 25 per cent of penicillia, and of a few species of fungi imperfecti.

They later (1944) extended this study to include over 1000 different species of fungi of various genera. Their results are summarized in table II.

TABLE II

Antibiotic Production by Fungi

Order or Genus	Total Number Examined	Active (%)	Weakly Active (%)	Inactive (%)
Phycomycetes	30	....	....	100
Ascomycetes	20	....	....	100
Aspergillia	150	30	20	50
Penicillia	200	20	30	50
Basicomycetes	730	10	20	70

Atkinson (1943) has qualitatively examined 18 species of penicillia for antibiotic activity. All gave positive tests for the presence of either penicillin or penicidin.

The group of bacteria classified as the actinomycetes represent a useful source of antibiotic substances. Gratia and Dath (1924) demonstrated the lytic effect of actinomyces upon dead bacteria. This effect was studied more extensively by Welsch (1937), who designated the collection of properties associated with the effect the 'actinomycetin' type of activity. Characterization of a specific agent from actinomyces was accomplished by Waksman and Woodruff (1941) with the isolation of actinomycin A. Welsch (1942) later examined 275 strains of actinomyces collected at random for lytic and bacteriostatic action. It was found that lytic activity for living gram-positive bacteria and dead bacteria of all types was widespread; inhibition of gram-negative forms was exceptional. The protein nature of "actinomycetin" (Welsch, 1941) suggests a relationship to lysozyme, although the former has no effect upon *Micrococcus lysodeikticus.*

With the exception of bacteriophage and the actinomyces group antibiotic products of bacterial origin appear to be less ubiquitous than those originating from fungi. This property, on the basis of available data, appears to be limited to certain spore-forming types and to a few cocci. Aside from the substances listed in table I, only two other reports of bacterial

production of antibiotics have appeared. In 1940, Hoogerheide described the preparation, from cultures of a soil bacillus of a material which was effective in inhibiting the encapsulation of Friedlander's bacillus. The crude preparation proved to be bactericidal for a number of gram-positive organisms. Subsequent investigation (McDonald, 1940) resulted in the isolation of a material which was relatively ineffective in capsule inhibition, but which proved to be highly potent as a bacteriostatic agent. It was shown that a number of spore-formers, among them *Bacillus subtilis*, *Bacillus megatherium*, *Bacillus mesentericus*, and *Bacillus cereus* were sources of similar agents. Tishler, Stokes, Trenner, and Conn (1941) established the identity of Hoogerheide's compound with Dubos' gramicidin.

An investigation into the cause of the inhibition of starter cultures used in cheese-making led Mattick and Hirsch (1944) to isolate an inhibitory substance from cultures of a streptococcus identified as belonging to Lancefield's group N. The polypeptide or protein nature of the inhibitor, as well as its source, suggests a possible relationship to diplococcin (Oxford, 1944).

Although indications are that antagonistic substances are produced by yeasts (Waksman, 1945), there appears to be no evidence that even partially purified fractions have yet been prepared.

A low yield of an antibacterial substance from suspensions of *Chlorella vulgaris* and *Chlorella pyrenoidosa* grown on a simple medium in the presence of light and CO_2 was reported by Pratt and others (1944). Although only a few organisms were tested for their sensitivity to the active material (which was named chlorellin), they represented a wide variety of bacterial types. Both gram-positive and gram-negative organisms were affected.

Perhaps the first indication of the occurrence of antibiotic substances in green plants was provided in 1940 by a group of Italian workers (quoted in a note in Lancet, 1940, I, 1184) who showed that honey possessed definite inhibitory power for *Escherichia coli*, *E. typhi*, *Shigella dysenteriae*, *Staphylococcus aureus*, and *Bacillus anthracis*. Their data indicated that

the antibiotic was actually present in the nectar source, ruling out the possibility of the production of the active substance by the bee. Osborn (1943) found antibacterial action in 2300 species of plants distributed among 63 genera. It was found that the antagonism for E. *Coli* generally paralleled the inhibition of *S. aureus*. Later Heatley (1944) isolated a crystalline material of relatively low antibacterial activity from *Crepis taraxacifolia*, which he termed crepin, and described some of its physical and chemical properties. A considerably more potent antibiotic, allicin,* has been isolated by Cavallito and Bailey (1944A) from *Allium sativum*. Unlike crepin, allicin appears to be equally effective against gram-positive and gram-negative forms. Allicin is one of the few antibacterials which is liquid at ordinary temperatures. More recently, Cavallito, Bailey and Kirchner (1945) have crystallized an antibacterial substance from the leaves of the common burdock, *Arctium minus*.

A recent survey of the antibacterial properties of 150 species of plants in Indiana revealed that only about 15 of those tested possessed significant bacteriostatic properties, and that the activity was very low in nearly all cases (Sanders, Weatherwax, and McClung, 1945).

Some proteins of plant and animal origin are known to have antibacterial properties; wheat germ contains such a substance. Some of the simple basic proteins such as salmine and histone are rather markedly bactericidal (Miller, Abrams, Dorfman and Klein, 1942). One of the most interesting of the antibacterial proteins is lysozyme. Discovered by Fleming in 1922 as a component of human nasal mucosa, tears, and saliva, this substance (or closely related compounds) was subsequently found to be widely distributed in many animals. Its ubiquitous nature suggests an important role as a natural defense against infection.

*In order to avoid confusion with certain medicinal products, Cavallito later withdrew the name allicin. Inasmuch as no substitute has as yet been proposed, the term allicin will be used throughout this review.

The Chemical Nature of Antibiotic Substances

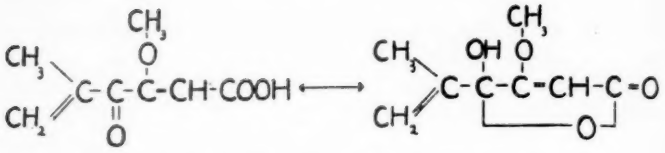
The antibiotics are a group of substances which exhibit analogous effects upon microorganisms; the members of the group, however, represent a wide range of substances of unrelated chemical constitution. Included are simple α, β-unsaturated lactones, quinones, polypeptides, and low molecular weight proteins. Some chemical correlation is apparent between the agents formed by related species of microorganisms; penicillic acid, clavacin, fumigatin, and citrinin, all of which are α,β-unsaturated ketones, are formed by more or less related groups of fungi; streptomycin and streptothricin, similar complex bases, are both produced by actinomycetes; and gramicidin, tyrocidine, and gramicidin-S, all polypeptides, represent metabolic products of spore-forming bacilli. Aside from some extensive studies which have been made on the relationship between antibacterial activity and structure for the quinones (e.g., Armstrong, Spink, and Kahnke, 1943), it is not yet possible adequately to relate structure or structural elements to antibiotic activity.

In this section there will be summarized the data upon which the knowledge of the structures of some of the antibiotics is based, together with information concerning those substances for which structural identification is incomplete.

Penicillic Acid

It will be recalled that penicillic acid was first described in 1913, by Alsberg and Black, as a metabolic product of *Penicillium puberulum*. Its structure was established in 1936 (Birkinshaw, Oxford, and Raistrick).

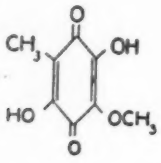
Penicillic acid is structurally perhaps the simplest compound which may be included among the antibiotics. Oxford, Raistrick, and Smith (1942) have suggested that the keto-acid (I) probably exists in tautomeric equilibrium with the unsaturated lactone (II):



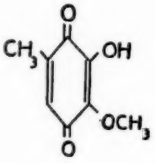
TAUTOMERIC FORMS OF PENICILLIC ACID

Spinulosin and Fumigatin

These two compounds may be considered together since they are closely related structurally, both being derivatives of toluoquinone. Although both substances had been isolated earlier, their structures were not established until 1938 (Anslow and Raistrick).



SPINULOSIN



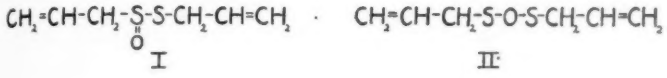
FUMIGATIN

It is perhaps surprising that the introduction of a second hydroxyl group into the fumigatin molecule results in a compound of rather markedly decreased activity (see Fig. 1). In a study of the effect of introducing various substituent groups upon the antibacterial activity of quinones, Oxford (1942) found that changing from a methoxy group to a phenolic hydroxyl at the two position exerted a profound effect. The 2-hydroxy analogues of spinulosin and fumigatin are much less active than the original compounds.

Allicin

Allicin was the name originally applied to the antibacterial principle of the garlic clove, *Allium sativum* (Cavallito and Bailey, 1944A). The possibility that the antibacterial activity might be due to the unsaturated compounds which can be derived from garlic (diallyl disulfide, acrolein, or crotonalde-

hyde) was ruled out by appropriate experiments. The empirical formula is $C_6H_{10}OS_2$, and its reactions indicate that one of two possible structures is likely (Cavallito, Buck and Suter, 1945).



I II

POSSIBLE STRUCTURES FOR ALLICIN

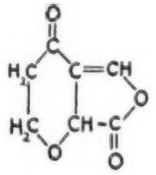
The relatively high water solubility (2.5%) favors structure I

Allicin is a colorless oil with a characterictic garlic-like odor. The pure preparation is very unstable, although dilute aqueous solutions, and the untreated plant tissue, show only slight loss of activity. Cavallito, Bailey and Buck (1945) suggest that allicin exists in the plant as an inactive precursor which is enzymatically activated in the presence of water to allicin itself.

Clavacin

As has been previously noted, clavacin has variously been termed clavatin, claviformin, and patulin. The structural evidence has been well summarized by Raistrick, Birkinshaw, Michael, Bracken, and Hopkins (1943).

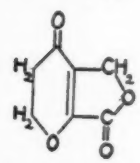
Clavacin is a white crystalline compound obtained in high yield (1 gram per liter of culture) from *Penicillium patulum*. It melts at 111°. Elemental analysis and molecular weight determinations established the empirical formula $C_7H_6O_4$. It is a strong reducing agent, optically inactive, and forms a 2, 4 dinitrophenylhydrazone. Although clavacin is a neutral substance, it is irreversibly inactivated by alkali. Raistrick et al., suggests the formula:



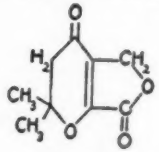
CLAVACIN

Bergel and his co-workers (1943) have suggested that the above structure does not fit completely the detailed analytical data.

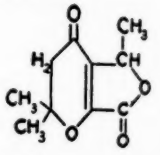
Puetzer, Nield, and Barry (1945) have synthesized a clavacin isomer (I), as well as two homologues (II and III).



ISO-CLAVACIN
(I)



1, 1, DI-METHYL
ISO-CLAVACIN
(II)

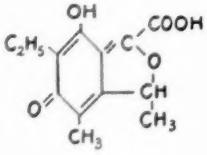


1, 1, 7 TRI-METHYL
ISO-CLAVACIN
(III)

I is slightly bacteriostatic for *Staphylococcus aureus*. II and III are essentially inactive. Apparently, rearrangement of I into clavacin cannot be effected.

Citrinin

Raistrick and Smith (1941) found that the metabolism solution of *Penicillium citrinum* shows a relatively low order of antibacterial activity. Upon acidification, the active material is precipitated in relatively pure form. Citrinin is a yellow crystalline solid melting at 170–171° which gives an intense brown color with ferric chloride. The existence of two acid groups, pK 5.7 and 9.9 may readily be demonstrated. The empirical formula is $C_{13}H_{14}O_5$. Raistrick and Smith have suggested the formula:



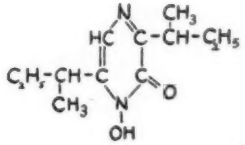
CITRININ

Citrinin has also been isolated from cultures of *Aspergillus candidus* (Timonin, 1942). Particular interest in this antibiotic arises from the fact that high yields (3.8 grams per liter) are readily obtained, and because purification is very

simple. Its stability to autoclaving is likewise an important factor.

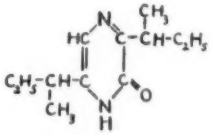
Aspergillic Acid.

Aspergillic acid was isolated from cultures of *Aspergillus flavus* by White and Hill (1943). Following adsorption of the active material upon charcoal, elution with ether, and resolution in $NaHCO_3$, the acid is precipitated with HCl and recrystallized from ethanol. Even pure preparations show a very wide melting range, from 84 to 116°, generally attributed to polymorphism. Analysis gives 64.4% carbon, 9.15% hydrogen, 12.8% nitrogen, and no sulfur or phosphorous. The molecular and equivalent weights are about 220, and hence the empirical formula is $C_{12}H_{20}N_2O_2$. There are no amino acids or peptide bonds. Further study (Dutcher and Wintersteiner, 1944) revealed that the acid group has pK=5.5, and that aspergillic acid is also a weak base, forming a well-defined hydrochloride. It is optically active. Dutcher and Wintersteiner have proposed the formula:



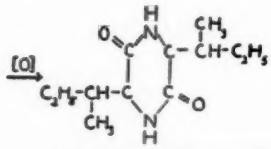
ASPERGILLIC ACID (I)

Evidence for the probable correctness of structure I comes from the fact that desoxyaspergillic acid



DESOXYASPERGILLIC ACID (II)

can be oxidized to:



OXIDATION PRODUCT OF DESOXYASPERGILLIC ACID (III)

III may be recognized as the cyclic anhydride of isoleucine, from which it has been synthesized.

Gliotoxin.

Gliotoxin was found as a metabolic product of fungi of the *Gliocladium* and *Trichodermata* genera by Weindling in 1934. Some evidence as to its chemical nature was adduced by Weindling, but nearly all of the detailed information relating to its structure has been provided by Johnson and his co-workers (Johnson, Bruce and Dutcher, 1943; Bruce, Dutcher, Johnson and Miller, 1944; and Dutcher, Johnson, and Bruce, 1944).

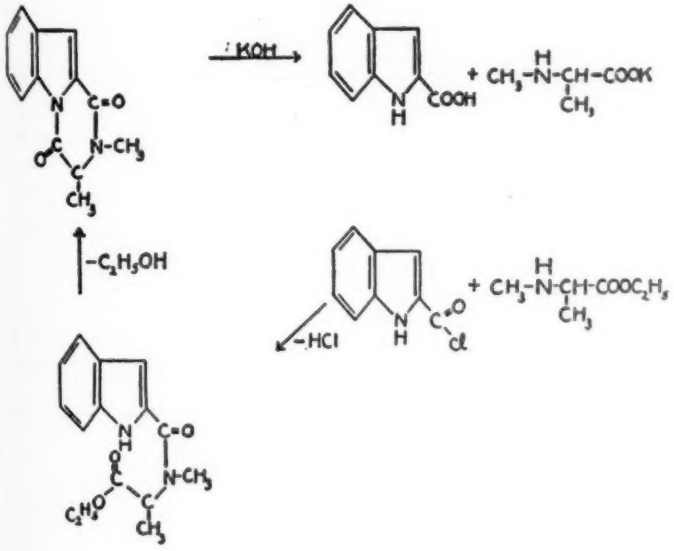
A four-day shaken culture of *Gliocladium fimbriatum* is extracted with chloroform, the active material being found in the chloroform layer. The solvent is evaporated, the residue dissolved in methanol, decolorized with charcoal, and crystallized from methanol. Yields are relatively low, about 3.0 grams being obtained from 60 liters of fluid.

Gliotoxin crystallizes in colorless monoclinic plates, m.p. 221°. Elemental analysis reveals the following composition: 48.1% carbon, 4.97% hydrogen, 8.2% nitrogen, and 19.4% sulfur. In ethylene bromide the apparent molecular weight is 314, and the probable formula is thus $C_{13}H_{14}N_2O_4S_2$. Gliotoxin is optically active, is stable in acid solution, and readily decomposes in alkali. Alkali decomposition results, in five days at room temperature, in complete loss of optical activity. Absorption spectrum studies are suggestive of the presence of an indole nucleus.

The sulfur in gliotoxin is in an extremely labile form. Oxidizing agents, under very mild conditions, form sulfate, and

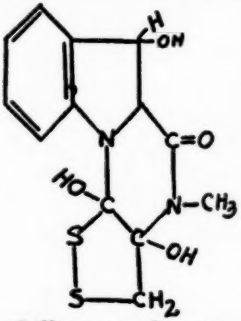
reducing agents easily convert the sulfur to hydrogen sulfide. The latter substance is also formed when gliotoxin is refluxed with dilute alkali, along with methylamine and free sulfur. There are no alkoxy groups in the molecule, and tests for a reactive carbonyl group are negative. Zerewitinoff determination of the number of active hydrogens indicates that two, or possibly three are present. Acylation with benzoyl-, p-brombenzoyl-, or p-nitrobenzoyl chlorides introduces two such groups, but upon acetylation a mono-acetyl derivative is formed.

Following treatment with alkali, the non-volatile residue has been shown to contain indole-2-carboxylic acid. Upon reduction with hydriodic acid and phosphorous, gliotoxin is converted into a neutral compound, $C_{13}H_{12}N_2O_2$, with H_2S and H_2O as by-products. The neutral C_{13} derivative appears to be a lactam, and can be further split (by alkali) into indole-2-carboxylic acid and a four-carbon fragment $C_4H_9NO_2$. Finally, by condensing indole-2-carboxylic acid with N-methyl alanine ethyl ester, and cyclizing, the sulfur-free derivative of gliotoxin is formed. The reactions involved are:



The compound $C_{13}H_{12}N_2O_2$ is thus 2,3 dimethyl, 1,4 diketo tetrahydropyrazino (1,2 α) indole. It differs from gliotoxin by two sulfur atoms and the elements of hydrogen peroxide.

In a paper published very recently, Dutcher, Johnson, and Bruce (J. Am. Chem. Soc., 67, 1736–1745, 1945) suggest the following structure as probably representing the true constitution of gliotoxin. The β-hydroxyindoline nucleus is presumed to undergo dehydration very readily under the influence of acids or bases.

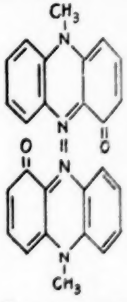


PROVISIONAL STRUCTURE FOR GLIOTOXIN

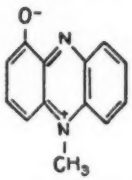
This is the structure suggested by the Lilly research group (in *Research Today*, vol. II, no. 2, Spring, 1945)

Pyocyanine

Cultures of *Pseudomonas aeruginosa (Ps. pyocyanea)* have been shown (Schoental, 1941) to possess antibacterial activity due primarily to three factors. Two of these are pigments derived from phenazine. The characteristic blue pigment, known as pyocyanine, has been the subject of an intensive chemical investigation by Wrede and Strack (1929), who have shown that the data require the structure:



PYOCYANINE

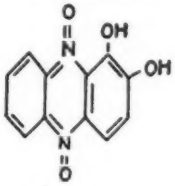
The reduction product,  has been synthesized.

REDUCTION PRODUCT OF PYOCYANINE

In addition to pyocyanine, cultures of *Pseudomonas aeruginosa* also contain a yellow pigment, ∝-oxyphenazine. The antibacterial activities of these substances, as well as that of "pyocyanase" have been studied by Schoental, but the data are limited.

Iodinin

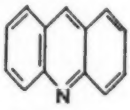
Closely related to pyocyanine is the antibiotic produced by *Chromobacterium iodinium*, a purple-bronze pigment known as iodinin. The structure, as established by McIlwain (1941), is:



IODININ

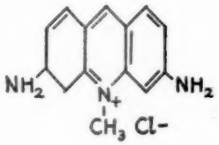
It is thus a dihydroxy, N,N′ dioxy phenazine. There is some doubt as to the positions of the hydroxyl groups. The ∝,β-arrangement is preferred by McIlwain.

The structures of the bacterial products mentioned above are particularly interesting in that a related group of synthetic compounds have found extensive pharmacological application. The acridine nucleus,



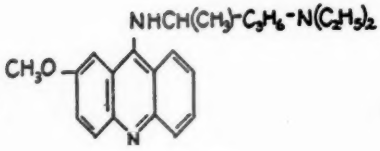
ACRIDINE

forms the basis of a number of important bactericidal, trypanocidal, and plasmodicidal compounds, of which trypaflavin



TRYPAFLAVINE

and atabrine



are typical.

ATABRINE

Apparently, no data is available relating to the possible effect of pyocyanine or iodinin on organisms other than bacteria.

Puberulic and Puberulonic Acids

Puberulic and puberulonic acids are metabolic products of *Penicillium puberulum* and *Penicillium aurantio-virens* respectively. They were first described by Birkinshaw and Raistrick (1932); subsequently their antibacterial properties were described by Oxford, Raistrick and Smith (1942). Both are relatively weak antibacterials. The empirical formula of puberulic acid is $C_8H_6O_6$, and that of puberulonic acid is $C_9H_4O_6$, but their structures remain unknown. Birkinshaw and Raistrick have shown that puberulic acid contains four acetylatable groups, and that the acidic properties persist after methylation or acetylation of two of them. The tetramethyl derivatives are, however, neutral. Barger and Dorrer (1934) presented evidence that puberulic acid contains a carboxyl group which is readily removed by decarboxylation.

Pyocyanase

Early studies upon the antibacterial activity of cultures of *Pseudomonas aeruginosa* (Emmerich and Löw, 1901) revealed the presence of a lytic fraction; because this lysis was thought to be due to an enzymatic reaction, the active material was labeled pyocyanase. Subsequent studies have indicated that pyocyanase is actually a mixture of lipoid substances, and the bacteriostatic activity is now generally conceded to be the attribute of free fatty acid components of the mixture (Waksman, 1945).

Crepin

The antibiotic formed by *Crepis taraxacifolia* is isolated by aqueous extraction of macerated plant tissue (Heatly, 1944). After removal of dissolved proteins, and further purification by adsorption, the active substance is crystallized from ether. Yields of 100 mg. of crystalline product from one kilogram of starting material are obtained.

Analytical data conform to the empirical formula $C_{14}H_{16}O_4$. The presence of at least two double bonds, probably conjugated, is indicated by reactions with bromine, permanganate, and hydrogen. Its slow solubility in alkali, with accompanying inactivation, suggests the presence of a carboxyl group masked by lactone formation. It is concluded that crepin, like some of the other antibiotics, is an unsaturated lactone.

The Antibiotic Principle of *Arctium minus*

An antibacterial inactive against gram-negative bacteria, but with some activity against gram-positive forms, was discovered in leaves of young plants of the common burdock, *Arctium minus*, by Cavallito, Bailey, and Kirchner (1945). It can readily be crystallized from hydrocarbon solvents. Analysis indicates the empirical formula to be $C_{15}H_{20}O_5$. Other properties suggest the presence of an unsaturated lactone moiety.

Fumigacin

As has already been indicated, the first preparations of fumigacin (Waksman, Horning and Spencer, 1942) contained nitrogen. This was later shown to be due to the presence of

gliotoxin. Fumigacin is nitrogen-free, and the analysis indicates an empirical formula of $C_{32}H_{44}O_8$ (Chain, Florey, Jennings, and Williams, 1943; Menzel, Wintersteiner, and Hoogerheide, 1944). This formula is in reasonable agreement with the value 510 obtained for the molecular weight by the Rast method, and agrees even more closely with the X-ray value of 562. One carboxyl group is present; this leads to the ready formation of a crystalline silver salt and a monomethyl ester. The compound shows no enolic or phenolic properties. No reducing groups are present, and the rapid inactivation by alkali with accompanying rise in levo-rotation is suggested as being due to the opening of a lactone ring. An aliphatic double bond is present, as shown by the ready absorption of one mole of bromine and the fact that fumigacin readily reacts with permanganate, but attempts to prepare a dihydro-fumigacin by catalytic hydrogenation were unsuccessful.

Chaetomin

On the basis of recent studies (Geiger, Conn and Waksman, 1944) chaetomin is apparently one of the most powerful antibiotics yet discovered. It is, unfortunately, inactive *in vivo* (Waksman and Bugie, 1944). Early preparations used the culture filtrate as a source; it was later found that the mycelium was much richer in chaetomin. Acetone extraction of the mycelium yields a non-crystalline product which melts over a narrow range (218–220°) and is apparently of high purity. Chaetomin is water insoluble, contains 54% carbon, 4% hydrogen, 13% sulfur, and about 10% nitrogen. There are no peptide bonds, tyrosine, or tryptophane. It is thermostable, and unaffected by acid; alkali causes inactivation.

Penicillin

Although penicillin has been subjected to more detailed study than any other antibiotic, available data relating to its chemical constitution are very limited. In 1942, the Oxford group published some fairly extensive reports of chemical investigations. A few similar studies were reported by American workers in the same year and in the early part of 1943.

For two years, however, practically no constitutional data have been recorded. In part this has been due to the recognition by various groups of investigators that even highly potent preparations are quite impure, together with more recent recognition that the active fraction consists of a mixture of compounds. Crystalline penicillin has, however, been available long enough to have overcome these difficulties. Doubtless the element of "military security" has contributed in some measure to the delay in dissemination of structural information. Unfortunately, other less scientific factors appear also to be involved. The available data are sufficiently extensive to be useful for the purposes of this review.

Although many techniques have been used for the purification of penicillin, some clues to its chemical nature are obtained from a consideration of the early techniques used. The filtered medium is acidified, and extracted with ether or amyl acetate. It can then be reëxtracted from the latter solution with buffer at pH 7. After clarification and a second distribution between solvents, the active material is adsorbed upon an appropriate adsorbent. Elution with either buffer or acetone results in a product of considerable increased purity, which may be further fractionated.

The early chemical data of Abraham and Chain (1942) were obtained using a preparation of the barium salt of penicillin containing approximately 500 units per milligram. (Crystalline sodium penicillin has a potency of about 1650 units per milligram.) Analysis of barium penicillin reveals the presence of 44.3% carbon, 4.85% hydrogen, and about 22% barium. There is apparently no phosphorous or sulfur. The nitrogen analysis of earlier preparations gave low and inconsistent results. However, when some penicillin fractions were subjected to sulfuric acid hydrolysis, traces of pyridine could be detected in the residue (Abraham, Baker, Chain, Florey, Holiday and Robinson, 1942), and when vigorous conditions are employed both Kjeldahl and Dumas determinations yield identical values of 4.13%. These data suggest either the formula $C_{23}H_{30}N_2O_9$ Ba or $C_{24}H_{32}N_2O_{10}Ba$, but do not, *a priori*, exclude the possible existence of a molecule of half the size of that indicated. It

was found that penicillin was an extremely hygroscopic substance (a property shared by its sodium and ammonium salts), freely soluble in water and in organic solvents. Aqueous solutions are fairly stable at pH 5.5–7.5 at room temperature and below, but are quickly and completely inactivated at 100°. Acid and alkali also effect rapid inactivation.

Penicillin is a strong dibasic acid with the two dissociation constants nearly identical (pK=2.4 and 3.5). At relatively high pH a new acid group is formed, with $pK_3=7.6$. Heating penicillin (barium salt) at 100° results in the evolution of one mole of CO_2 per gram atom Ba. The acidic dissociation constants are unchanged by this procedure, indicating that the carbon dioxide does not come from either of the original acid groups of the molecule. Reducing agents are generally more effective in the inactivation of penicillin than are oxidizing agents, and under the proper conditions hydrogen is absorbed in the proportion of 0.8 moles H_2 per gram atom of Ba.

Penicillin shows evidence for the existence of two acetylatable groups and at least five angular methyl groups. There are no ethoxyl or methoxyl groups. Although Abraham and Chain could not prepare penicillin methyl ester by the action of methyl iodide upon the silver salt, Meyer, Hobby, and Chaffee (1943) reported the preparation of the methyl, ethyl, butyl, and benzohydryl esters by reaction of free penicillin with the corresponding diazo compounds. More recently, the preparation and properties of penicillin benzyl ester, from free penicillin and phenyl diazo methane, has been described (Cavallito, Kirchner, Miller, et al., 1945).

Meyer, Chaffee, Hobby, Dawson, Schwenk, and Fleischer (1942) suggested that the empirical formula best conforming to their analytical results was $C_{14}H_{17}NO_6$, possibly containing one mole of bound water. However, diffusion constant measurements on penicillin (Frieden, 1945) suggested a molecular weight of about 500; this is in good agreement with the formula proposed by Abraham and Chain.

Although Abraham and Chain reported that the treatment of penicillin in organic solvents with ammonia resulted in inactivation, Meyer, et al., were able to prepare an active am-

monium salt by passing dry NH_3 into a chloroform-benzene solution of penicillin. When the ammonium salt was acylated, a crystalline derivative was obtained, which retained the major part of the original antibacterial activity.

Some information is available relating to the nature of the degradation products of penicillin. Catch, Cook, and Heilbron (1942) subjected the strontium salt of penicillin to the action of dilute acid and alkali. Either treatment produced a water soluble acid, an insoluble yellow pigment, acetaldehyde, and a seven carbon α,β-unsaturated aldehyde. The water soluble acid was possibly an amino acid or peptide. The pigment corresponds to the formula $C_{16}H_{20}O_5$. Upon treatment of penicillin with ammonia, a fluorescent product is formed.

After dilute sulfuric acid hydrolysis of penicillin, a deep blue-violet color with ninhydrin is obtained (Abraham, Chain, Baker, and Robinson, 1943). The intensity of the color was proportional to the antibiotic content, except for grossly impure preparations. Somewhat more than half the nitrogen in penicillin can be obtained in the Van Slyke apparatus after acid hydrolysis. This nitrogenous penicillin derivative was named penicillamine, and has been prepared in crystalline form as the hydrochloride. Its formula is $C_6H_{11}O_4N.HCl$ (this may be the hydrate). Penicillamine is optically inactive. The hydrochloride is a tribasic acid, and Abraham and his co-workers have suggested that the three acidic groups possibly are a carboxyl group, the substituted ammonium group, and a weakly acidic (enolic?) hydroxyl. The apparent dissociation constants are of such magnitude as to agree with this suggestion. Penicillamine is a strong reducing agent. When heated in acid solution with paranitrophenylhydrazine, a 20% yield of the paranitrophenylhydrazone of glyoxal is obtained.

Duffin and Smith (1943) have reported the formation of an unsaturated acid, called penillic acid, when penicillin is allowed to stand at pH 2 for a long time. The purified acid, like penicillamine, gives a positive ninhydrin reaction, but is optically active. It is fluorescent in ultraviolet light. It will be recalled that fluorescent products are also formed by the action of ammonia upon penicillin. The writer has found that, while

commercial preparations of penicillin slowly develop fluorescence when treated with ammonia or sodium hydroxide, the fluorescence developed with these reagents when crystalline penicillin is used is negligible.

So far as the writer is aware, no chemical studies dealing with crystalline penicillin have been reported.

In view of the fact that penicillic acid, clavacin, and other antibiotics resemble each other in possessing an unsaturated lactone grouping, it is of interest to point out that the studies summarized above suggest the presence of this structural moiety in penicillin as well. It is possible that the lactone ring involves the tautomeric form of a carbonyl group.

Subtilin

The antibacterial activity of ***Bacillus subtilis*** filtrates was first attributed to tyrothricin-like polypeptides which could be isolated from the metabolism solution. Jansen and Hirschmann (1944), however, showed that the crystalline polypeptide fraction was virtually inactive, and that the active fraction could best be extracted with aqueous ethanol from the cellular mass. The crude preparation possesses an activity about equal to that of tyrothricin upon some gram-positive forms; *Eberthella typhosa* is, however, unaffected. Subtilin apparently contains 5.7% nitrogen and a high proportion of phenolic residues (33% using tyrosine as a colorimetric standard). It is maximally stable at low pH and readily inactivated by heat at pH 7 and above. Sterilized solutions are also unstable to light.

On the basis of comparative diffusion rates in agar, the molecular weight of subtilin approximates that of penicillin more closely than it does that of tyrothricin.

Actinomycin

From a species of actinomyces *(Actinomyces antibioticus)* Waksman and Woodruff (1941) succeeded in isolating two antibacterials, actinomycin A and actinomycin B. Actinomycin A is primarily bacteriostatic, while actinomycin B appeared to be essentially bactericidal in action. The bacteri-

cidal activity of actinomycin B was evident only at high concentrations and varied widely with different preparations.

From cultures of *Actinomycin antibioticus*, actinomycin A is removed by extraction with ether. After evaporation of the solvent, the residue is extracted with petroleum ether to remove impurities. The residue from the petroleum ether treatment, which contains essentially all of the activity, is dissolved in benezene and purified by chromatographic adsorption upon alumina. After elution with acetone-benzene, crystallization from acetone-ether results in the deposition of red platelets, m.p. 250° with decomposition (Waksman and Tishler, 1942).

Analysis shows 59.01% carbon, 6.81% hydrogen, and 13.35% nitrogen. There is no phosphorous, sulfur, or halogen. About one-fourth the nitrogen appears to be in the form of methylated amino groups; at least one acetyl group is also present. Cryoscopic determination of the molecular weight gives values of 750–1000, depending upon the solvent. The probable formula is $C_{41}H_{56}N_8O_{11}$, or $C_{37}H_{50}N_7O_{10}$. Actinomycin is soluble in strong hydrochloric acid but not in alkali. It is readily reduced by stannous chloride, bisulfite, or hydrogen; the reduced product is reoxidized by air. At least two more acetyl groups are introduced by either reductive or non-reductive acetylation. Waksman concluded that actinomycin is a polyfunctional quinone, with the nitrogen present in weakly basic form.

Streptothricin and Streptomycin

Streptothricin (from the generic name streptothrix) is formed by *Actinomyces lavendulae;* streptomycin is a closely related compound found in cultures of *Actinomyces griseus.* Neither has thus far been obtained in a pure state. Crude preparations of streptothricin are precipitated by protein reagents, but the protein fraction is removed during the process of purification. It is soluble in water and acids, insoluble in hydrocarbon solvents. Crude preparations, possibly because of the presence of protein, are unstable to heat, but relatively pure fractions may be autoclaved without inactivation. No analytical data for either compound is available.

Proactinomycin

This material, product of a microorganism described as being of the genus proactinomyces, resembles in some of its properties the organic bases obtained from actinomyces. Proactinomycin is basic, being extractable by organic solvents from aqueous solutions at pH 10, is stable to heat at low pH, and is precipitated by picrolonic and picric acids. No data is available as to its elemental composition or approximate molecular size.

Penicidin

Penicidin, discovered by Atkinson in the metabolic fluid of a mold of the genus Penicillium, is active against gram-positive and gram-negative forms (Atkinson, 1942). Its ready dialyzability indicates a relatively low molecular weight, while its stability properties (stable at pH $\leq$ 7, destroyed by alkali) are evidence of its basic nature.

Gramicidin and Tyrocidine

The available data concerning the chemical natures of gramicidin and tyrocidine have been reviewed by Hotchkiss (1944). The original observations on the antibiotic activity of *Bacillus brevis* were made on growing cultures, but filtrates of autolyzed cultures were found to possess antibacterial action as well. Such filtrates are brought to pH 4.8, whereupon a precipitate appears. Alcohol extraction of the precipitate, followed by the addition of NaCl to the alcoholic extract results in the precipitation of an amorphous material of high activity. This substance is designated as tyrothricin, after the older generic name for *Bacillus brevis*, tyrothrix.

When tyrothricin is treated with a mixture of acetone and ether, some of it goes into solution. After evaporating the solvent, the acetone-ether soluble component can be recrystallized from acetone. This is gramicidin.

The acetone-ether insoluble fraction of tyrothricin, which is still bacteriostatic, can be redissolved in alcohol, whence it can be precipitated in crystalline form by the addition of HCl. The acid nature of the precipitate led Dubos to label it "graminic

acid," but it was later recognized that the "acid" was actually the hydrochloride of a weak base; the name graminic acid was replaced by the designation tyrocidine hydrochloride. (Gramidinic acid was the name originally given to a partially neutralized fraction of tyrocidine hydrochloride).

Crystalline gramicidin is a neutral substance containing C, H, N, and O. A positive biuret test and negative protein reactions indicate that the molecule is polypeptide in nature. Analysis of gramicidin for individual amino acid constituents, especially tryptophane, suggest a minimum molecular weight of about 550, while analysis of the acetyl and flavianic acid derivatives agree more closely with estimates of 1200–1300 and 3000 ± 300, respectively. Cryoscopic molecular weight determinations in camphor give results in the range 1350–1600; in phenol the cryoscopic molecular weight is about 900, while similar determinations in cyclohexanol give anomalous results ranging from 590–1220, depending upon the concentration (Tishler, et al., 1941).

Probably the most reliable method of estimating the molecular weight of gramicidin is by use of the isothermal distillation technique. In methanol solution, Tishler and co-workers (1941) found that the apparent molecular weight of gramicidin is 3100; similar studies in butanol were in substantial agreement, limiting the molecular weight to the range 2500–4500.

Elemental analysis of gramicidin gives 62.7% carbon, 7.5% hydrogen, and 13.9% nitrogen. There are no free carboxyl or free amino groups. Acid hydrolysates of gramicidin have been shown to contain tryptophane, leucine, valine, alanine, and glycine (Gordon, Martin, and Synge, 1943), as well as an unidentified hydroxy-amino acid not identical with any known compound (Hotchkiss, 1941).

Assuming a molecular weight of about 2800, and 30 nitrogen atoms to the molecule, Hotchkiss (1944), has suggested that gramicidin consists of 6 residues of tryptophane, 6 of leucine, 4 each of valine and alanine, and 2 each of glycine and the unknown aminohydroxy compound. Since no volatile fatty acids or alcohols have yet been identified, and all of the carboxyl and amino groups are masked, it must be concluded that gramicidin

is a cyclic polypeptide. The arrangement of the component amino acids in the polypeptide chain is largely a matter of speculation. Christiansen (1943) has isolated valylvaline from gramicidin hydrolysates; two of the valine residues must therefore be adjacent.

Particular interest in the configuration of the component amino acids of gramicidin was stimulated by the discovery (Lipmann, Hotchkiss, and Dubos, 1914) that gramicidin hydrolysates could be oxidized by d-amino acid oxidase. Nearly fifty per cent of the α-amino nitrogen of gramicidin hydrolysates could thus be liberated. d-Leucine and d-valine have been identified among the hydrolytic products. The tryptophane and alanine are both entirely of the l-configuration. The configuration of the hydroxyamino component is unknown; there is some evidence that the extent of d-amino acid oxidase oxidation of gramicidin exceeds that to be expected on the basis of the known contents of d-leucine and d-valine. While it is possible that some racemization occurs during the process of hydrolysis, care has been taken to use relatively mild conditions, and the tryptophane recovered was essentially optically pure.

Inasmuch as d-amino acids have been found only rarely in nature (Lipmann, Hotchkiss, and Dubos, 1941; Bovarnick, 1942), the occurrence of these "unnatural" isomers in antibacterial polypeptides poses an interesting question as to the relationship which these isomers bear to antibiotic activity.

Tyrocidine is apparently a more complex and more reactive molecule than gramicidin, in that it contains a greater variety of amino acids and shows the presence of free amino (or other basic) groups, and of a weakly acidic group. Elemental analysis of tyrocidine hydrochloride reveals that it contains 59.7% carbon, 6.9% hydrogen, 14.5% nitrogen, and 2.52% chlorine (Christiansen, Edwards, and Piersma, 1941). The values for the approximate molecular size are more satisfying than those for gramicidin; alkali titration gives an equivalent weight of 855, chloride titration indicates an equivalent weight of 1285, while from the easily hydrolyzable ammonia content the value 815 is obtained (Hatchkiss, 1941). A minimum molecular

weight of about 2500 is thus indicated. Tyrocidine yields from 7 to 10 per cent of its total nitrogen as amino nitrogen in the Van Slyke procedure. Amino acids which have been identified in tyrocidine hydrolysates include aspartic and glutamic acids, ornithine, proline, leucine, valine (Gordon, Martin and Synge, 1943), tryptophane (Hotchkiss, 1941), alanine, and phenylalanine (Christiansen, Edwards and Piersma, 1941). A polypeptide containing two molecules each of aspartic and glutamic acids, ornithine, tryptophane, proline, tyrosine, leucine, and valine, and three of phenylalanine has been suggested by Hotchkiss (1944). The two basic groups of the ornithine residues would be available for binding HCl, three of the dicarboxylic acids would be combined with ammonia as the amides, while the three moles of alkali required in alkali titration might be accounted for by the other carboxyl group together with the two phenolic hydroxyl groups of tyrosine. Tyrocidine is thus thought of as a cyclic polypeptide similar to gramicidin.

Gordon, Martin and Synge (1943) have provided evidence that the phenylalanine of tyrocidine is present as the d-isomer, while the other amino acids appear to be of the l-configuration. It is of considerable interest that *Bacillus brevis* is thus able to synthesize one polypeptide (gramicidin) containing d-leucine, while it simultaneously uses l-leucine in the synthesis of tyrocidine.

Gramidicin S

Following a systematic survey of soil bacilli in 1942, Gause and Brazhnikova (1944) isolated a strain with marked antagonistic properties, from cultures of which an antibiotic was subsequently isolated. Because of the similarity of the organism used to *Bacillus brevis*, although the active principle was different from either gramicidin or tyrocidine, they named their product "Soviet gramicidin," or gramicidin S. Isolation of the pure antibiotic is readily accomplished by acidification of the metabolism solution and extraction of the resultant precipitate with ethanol. When the solvent is evaporated, gramicidin S precipitates in crystalline form.

Gramicidin S melts at 268–270°. It contains 13.0% nitrogen, as compared with 14.8% for gramicidin and 14.3% in tyrocidine. Fourteen per cent of the total nitrogen of the molecule is liberated as amino nitrogen in the van Slyke apparatus. The biuret and ninhydrin reactions are positive, but typical protein reactions are negative. Gramicidin S and its hydrolysates appear to contain no tryptophane, histidine, tyrosine, phenylalanine, arginine, or histidine, and glutamic and aspartic acids are also absent. Proline and leucine have been identified as hydrolytic products. About one-fourth of the total nitrogen exists in basic amino acids, probably as ornithine. Cryoscopic determination of the molecular weight gives values in the range 1060–1340. Apparently no data regarding the configuration of the constituent amino acids is available.

Diplococcin

This substance was isolated by Oxford from cultures of a species of streptococcus. It is precipitated from metabolism solutions as the picrate, and the latter is then decomposed with acetic acid. Diplococcin appears to be a low molecular weight protein, giving nearly all of the typical protein reactions. Elemental analysis reveals the presence of about 14% nitrogen, but no sulfur or phosphorous. Color tests for arginine, tyrosine, and tryptophane are positive, as is the Molisch carbohydrate reaction. The antibacterial properties of diplococcin are stable to heat at pH less than 7, but heating in alkali inactivates the molecule. Although the general properties of diplococcin resemble those of lysozyme, there appears to be no true lysozyme activity.

Notatin

Reference has already been made to the work of Coulthard, Michaelis, and Short (1941), to Coulthard, et al. (1942), and to Kocholaty (1942) upon the second antibiotic of *Penicillium notatum*. It was early recognized that notatin (penicillin A, penicillin B, and penatin are synonyms) was protein in nature. The most extensive reports dealing with the preparation and

properties of this protein antibiotic are those of Roberts, et al. (1943) and of Van Bruggen and his co-workers (1943).

Notatin is adsorbed from the culture medium upon benzoic acid. The adsorbent is then dissolved in acetone, whereupon the protein is precipitated. The precipitate may then be further purified by solution in water, followed by reprecipitation with acetone. An alternative purification procedure involves precipitation with uranium acetate, which appears to give a purer product.

When dry, or in solution at pH 3–6.8 notatin is quite stable. It is light yellow in color, soluble in water and insoluble in lipoid solvents. Typical protein precipitants (trichloroacetic acid, tungstic acid, etc.) precipitate the active substance, as do ammonium sulfate and magnesium sulfate at the proper concentrations. Notatin is non-dialyzable and gives the typical protein color reactions. A positive nitroprusside reaction was also obtained, indicating the presence of reactive sulfhydryl groups. Of a number of proteolytic enzymes tested, only papain inactivates.

The purest notatin preparations yet obtained retain their yellow color. When the yellow component is separated, as by methanol or salt extraction, or by prolonged dialysis, notatin is irreversibly inactivated. Van Bruggen and his co-workers have suggested that the yellow component is probably flavine adenine dinucleotide, and functions as the prosthetic group of the enzyme.

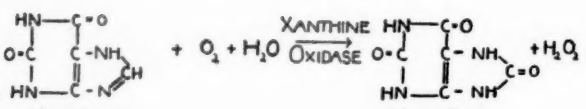
The mechanism of action of notatin is of considerable interest. It was early noted that notatin was equally effective against nearly all types of bacteria tested. Glucose and aerobic conditions are required for the demonstration of its antibacterial properties. Glucose can be replaced by galactose and xylose (with diminished activity), but not by other sugars. Under anerobic conditions in the presence of glucose, notatin decolorizes methylene blue, and the aerobic disappearance of glucose in the presence of notatin is accompanied by the formation of acid. This has been identified as gluconic acid; another product of the reaction is hydrogen peroxide. Finally, the activity of notatin is inhibited by serum.

These facts are adequately explained by assuming that notatin functions as a glucose oxidase, or, more correctly, as a glucose aerodehydrogenase, according to the reaction:

$$C_6H_{12}O_6 + O_2 + H_2O \xrightarrow{\text{NOTATIN}} C_6H_{12}O_7 + H_2O_2$$

The actual bactericidal agent is thus hydrogen peroxide. Schales (1943) has shown that the effects of notatin can be duplicated by H_2O_2 if the concentration of the latter is maintained at a level of 0.007%. Furthermore, the relative effects of glucose and galactose when used as substrates can be correlated with their respective rates of oxidation. The inhibitory effect of serum is explained as being due to the decomposition of H_2O_2 by catalase.

Unique confirmation of the above hypothesis has been provided by Green and Pauli (1943). These workers reasoned that if the activity of notatin as an antibiotic was due to the production of peroxide, other oxidases known to produce H_2O_2 ought to function similarly. This idea was tested with the enzyme catalyzing the transformation:



The xanthine oxidase can be obtained from milk, and media containing xanthine are necessary for its action as an antibiotic. At dilutions of 1:100,000,000 this "synthetic antibiotic" is effective against a wide variety (but not all) of microorganisms. Its antibacterial activity is reversed by catalase.

Lysozyme and Other Proteins

In 1922 Fleming described a potent bacteriolytic agent originally obtained from human nasal mucosa. The activity was displayed, at high dilutions, in both broth and agar. He described in detail some of the physical and chemical properties of lysozyme, and mentioned its occurrence in tears, serum, sputum, and peritoneal fluid in humans, as well as in some

other animal species. He also found that egg-white was an excellent source. Later, Florey (1930) conducted an extensive survey of the relative lysozyme contents of the tissues of a number of animals.

Most of the studies relating to the chemical constitution of lysozyme have been carried out using egg-white as the source. Meyer, Thompson, Palmer and Khorazo (1936) described one of the first attempts at purification. Roberts, Maegraith, and Florey (1938) compared with chemical constitutions of purified lysozyme from egg-white, cat, and human saliva. Other chemical data have been presented by Roberts (1938), Abraham (1939), and by Alderton, Ward, and Fevold (1945).

Various methods of purification have been used. Acetone precipitation of the active fraction is ordinarily the first step. The precipitate is redissolved in slightly acid alcohol and reprecipitated with an excess of alcohol after removal of the acid. Although most lysozyme preparations are amorphous solids, the preparation of a crystalline product has been reported. Comparison of the potencies of lysozyme prepared by different workers is rendered difficult by the fact that different methods of testing have been employed. Meyer, Thompson, Palmer, and Khorazo state that their crystalline preparation was lytic for *Sarcina lutea* at dilutions of 1:32,000,000. Most other products have activities of 1:2,000,000 to 1:5,000,000 as measured by lysis of *Micrococcus lysodeikticus*.

Lysozyme is a basic substance containing about 15% nitrogen, small amounts of sulfur and phosphorous, and a variable ash content. Protein reactions are positive, but there is no indication that carbohydrate is present. Various methods of molecular weight determination give values in the neighborhood of 18,000. The molecule is isoelectric at pH 10.5. Amino acids so far identified in lysozyme hydrolysates include arginine, lysine, cystine, tyrosine, and histidine. Assuming a molecular weight of 18,000, there appear to be nine free amino groups, nearly all of which are the ϵ-amino groups of lysine.

Comparison of lysozyme obtained from different species has been made on both chemical and immunological grounds. Differences in the nitrogen and sulfur contents of egg-white and

cat lysozymes have been reported by Roberts, Maegraith, and Florey (1938). More impressive, perhaps, are the data which indicate that egg-white lysozyme can function as an antigen for the rabbit, although lysozyme has been found in rabbit tissues (Roberts, 1938), and the reports that egg-white, cat, and human lysozymes can be differentiated immunologically (Roberts, Maegraith, and Florey, 1938).

In view of the fact that egg-white is a rich source of the "antibiotin" factor, avidin (Wooley and Longsworth, 1942), studies have been made on the relationship of these two proteins. Both are apparently basic proteins, of approximately equal isoelectric points, but the molecular weight of avidin appears to be much larger than that of lysozyme (about 70,000). Meyer (1944) has reported that avidin preparations show lysozyme activity proportional to their avidin content, and that the addition of biotin enhances lysozyme activity. Similar results were obtained by Laurence (1944). Both these workers suggested that lysozyme was actually the avidin-biotin complex. However, Alderton, Lewis, and Fevold (1945) after a study of purified lysozyme and avidin, concluded that the two substances were unrelated, although chemically they are quite similar.

Epstein and Chain (1940) have considered the mechanism of lysozyme action. According to them, lysozyme functions as a polysaccharidase, breaking down a bacterial polysaccharide into an N-acetyl amino hexose and a ketohexose. The polysaccharide substrate has been found in all bacteria sensitive to lysozyme, and all lysozyme preparations possess the ability to hydrolyze it.

A number of references to the antibacterial activity of proteins other than lysozyme have appeared in the literature. The presence of a bactericidal and bacteriostatic protein in wheat germ has been demonstrated (Stuart and Harris, 1942). While studying the synergic effect of certain basic proteins upon the sensitivity of gram-negative bacilli to tyrothricin, Miller, Abrams, Dorfman and Klein (1942) found that salmine sulfate and liver histone were essentially bactericidal for some microorganisms. The effect was particularly pronounced for gram-

negative forms. Miller and his co-workers, on the basis of the similarity between these bactericidal proteins and gramicidin and tyrocidine, have suggested that relatively simple polypeptide configurations might serve as the basis for a large number of antibacterials.

Chlorellin

Essentially no analytical data are available respecting chlorellin, the antibiotic of *Chlorella vulgaris* and *Chlorella pyrenoidosa*. Its chief interest lies in the fact that it can be produced on a very simple medium, only inorganic salts, light, and CO_2 being required, and thus may have some economic advantages.

Biological Activity of the Antibiotics

Any attempt quantitatively to compare the effects of the various antibiotic substances must take into consideration a number of factors which render such a comparison difficult. It has generally been the custom of workers in this field to designate the effectiveness of a given antibacterial in terms of the dilution (reciprocal of the concentration in grams per ml.) which just inhibits the growth of the organism being studied. This may be referred to as the maximum *bacteriostatic* dilution, and though the concept is an arbitrary one, it is justifiable in view of the fact that most clinical procedures involving antibiotic use are predicated upon bacteriostatic rather than upon bactericidal action. It should be borne in mind, however, that any bacteriostatic agent is bactericidal in sufficiently high concentration, and that the antibiotics differ sharply in the ratios of their bacteriostatic and bactericidal concentrations.

In this review, only bacteriostatic effects have been considered, partly because far more data is available on this point.

One of the chief difficulties involved here is that little consistency exists among the conditions which various investigators have employed in the study of antibacterial effects. Some workers prefer to use broth cultures of susceptible organisms, while others favor solid media. In part, this is unavoidable, due to the wide variation in the cultural characteristics of

bacteria, but comparison of the results obtained by different investigators may sometimes be of questionable validity. Fortunately, however, the bulk of the available evidence indicates that most of the antibiotics differ only slightly in activity as determined by either the broth or the agar method.

The antibiotic activity of many substances is dependent upon the constitution of the medium employed. Notatin, while highly active in glucose-containing medium, is almost completely inactive when this substance is absent. The effects of tyrocidine and gramicidin are markedly influenced by the presence of peptone. It is therefore hardly surprising that the results obtained in different laboratories reveal considerable discrepancy.

Although most antibiotics are not influenced, within wide limits, by the number of organisms present, there appear to be a few the effectiveness of which diminishes when large inocula are used. Diplococcin is an example.

One of the most important variables in bacteriostatic tests is the duration of the experiment. Extended growth periods may sometimes give ambiguous results, either by allowing the development of resistant forms, or by permitting the decomposition of a somewhat unstable test material. Ordinarily, this factor can be controlled by allowing the experiment to continue only as long as is necessary for an untreated culture to show abundant growth.

Finally, an extremely important factor underlying the divergent results obtained by different workers is that of strain variation. It has been shown (Watson, 1944) that naturally occurring strains of *Streptococcus pyogenes* may show variations of several thousand fold in their sensitivity to penicillin, and it is likely that strain variation in response to antibiotic action exists, in greater or lesser degree, among all bacterial species.

It is nevertheless possible to summarize systematically the available data relating to the antibacterial effects of most of the antibiotics. In order to facilitate comparison, the data have been collected in a "bacteriostatic profile" (Figure 1). The height of the column representing the effect of the substances

indicated upon the various microorganisms listed is proportional to the quantity log D–2, in which D is the minimum bacteriostatic dilution (in ml. of broth or agar per gram) necessary to inhibit growth. This method is chosen so as to be able to include substances of widely different activities on the same chart; the base line represents a dilution of 1:100 as a space-conserving measure. No distinction has been made between results in broth and agar. If data is available relating to the effect of strain variation, the total column height, solid and stippled, indicates the sensitivity of the least resistant strain, while the solid portion represents the dilution effective against the most resistant variety. Some of the data are approximate only, and in such cases a jagged peak is used to indicate this fact.

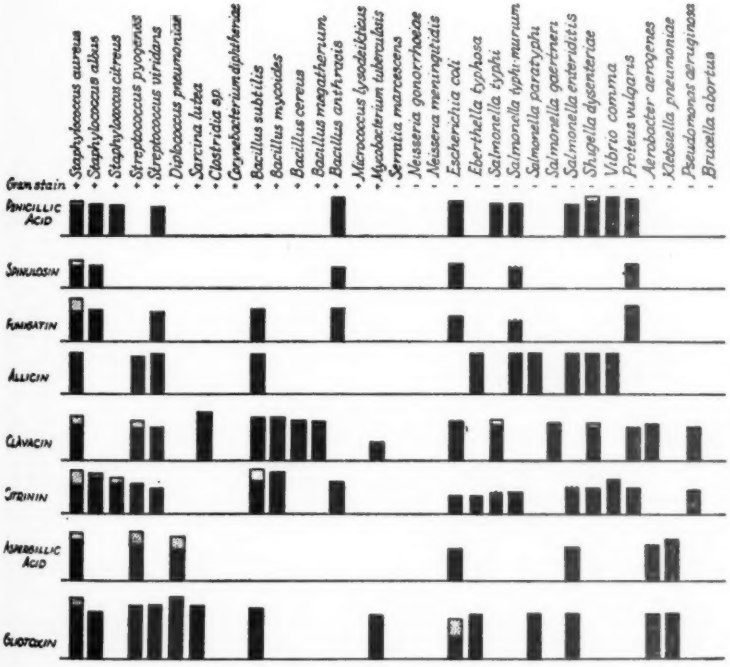


FIGURE I

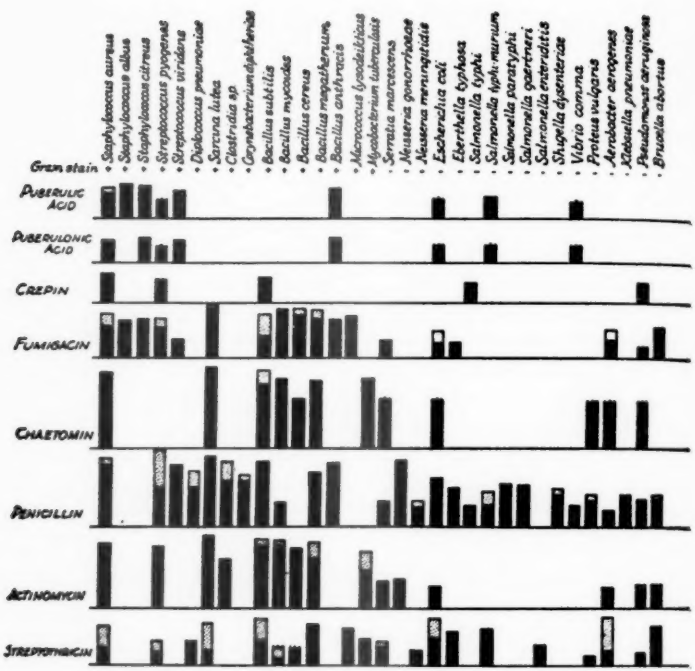


FIGURE I *(Continued)*

The data upon which Figure 1 is based were obtained from the original literature, supplemented where possible with additional data from Waksman (1945). The list of microorganisms includes nearly all those which have been studied, but a few species for which the data are fragmentary are not included. The vertical heading "*Clostridia* sp." includes *Clostridium welchii, Clostridium oedematiens, Clostridium septique,* and *Clostridium tetani;* apparently species as well as strain variation within this genus is minimal.

The data for penicillic acid, citrinin, spinulosin, fumigatin, puberulic and puberulonic acids, gliotoxin, crepin, fumigacin, aspergillic acid, actinomycin A, gramicidin, tyrocidine, and gramicidin S refer to the crystalline compounds. The extensive survey of the bacteriostatic properties of chaetomin reported

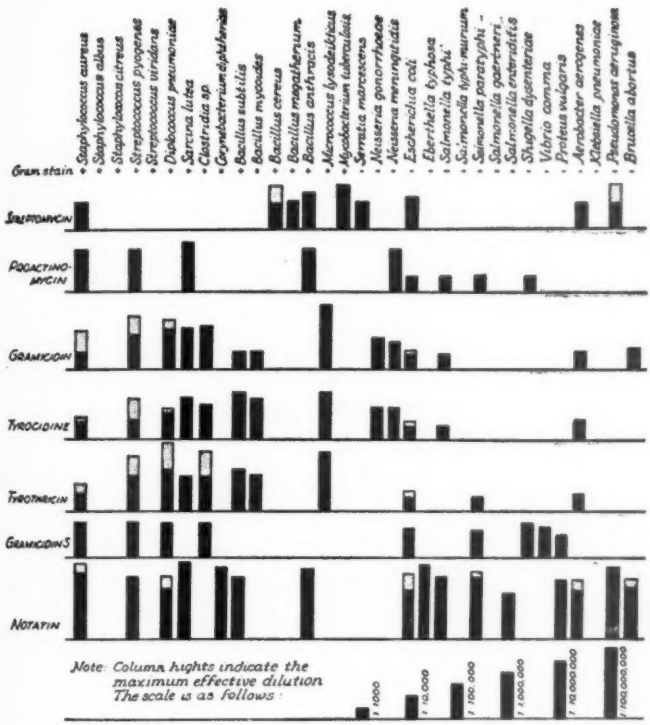


FIGURE I *(Concluded)*

by Waksman and Bugie (1944) referred to an impure preparation; when chaetomin was later crystallized (Geiger, Conn, and Waksman, 1944) it was found that the crystalline material was from 500–5,000 times as active as the earlier preparation depending upon the organism used for comparison. Since this second report included only a few species, the values for chaetomin in Figure 1 are those given in the first paper multiplied by the conservative factor 1,000 to give a closer approximation to the properties of the pure compound. Diplococcin, proactinomycin, streptothricin, streptomycin, and notatin were non-crystalline preparations, but probably of fairly high purity. The data for clavacin include those for the earlier non-crystal-

line fractions as well as for the crystalline compound; there appeared to be no significant differences between the activities of the two forms. Allicin is an oil at room temperature; the preparation used probably contained little inert material. The values for penicillin were corrected from the stated potency, using the figure 1650 Oxford units per mg. as the potency of pure penicillin. Although tyrothricin is not a pure compound, its expanding therapeutic use, as well as its apparent constancy of composition, affords sufficient justification for including it in this list.

Reference to Figure 1 makes it plain that the conventional distinction between gram-positive and gram-negative organisms is a relative one. The genus *Neisseriae*, although gram-negative, is highly susceptible to penicillin, while *Bacillus mycoides* is an example of a gram-positive form which is less sensitive to the action of this agent than some gram-negative ones. It should be noted that while penicillin is generally considered to be ineffective against gram-negative bacteria, it is actually one of the most potent with respect to these forms. It is only by contrast with the highly sensitive staphylococci and streptococci that its activity can be considered low.

The bacteriostatic profile of notatin is in keeping with existing theories of its mode of action. Compared to some of the other antibiotics species and genus variation is almost nonexistent. The limited data for gramicidin S are sufficient to show that it is distinctly different from either gramicidin or tyrocidine.

Of considerable interest, especially from a clinical standpoint, is the sensitivity of the various spirilliform organisms to the antibiotics. The sensitivity of *Treponema pallidum in vitro* to a number of antibiotics has been studied by Dunham, Hamre, McKee and Rake (1944). The criterion for effectiveness was immobilization of the actively motile organisms in a period of two hours. Their results are summarized in Table III.

The significance of these results as far as the clinical usefulness of antibiotics in the treatment of syphilis is concerned is uncertain. Unquestionably the reproductive activity of *Treponema pallidum* is sensitive to far lower concentrations of penicillin

TABLE III

Immobilization of Treponema Pallidum by Antibiotics

Compound	Dilution Used	Per cent of Spirochetes Immobilized in Two Hours
Penicillin	1:1,200	50
Aspergillic Acid	1:8,000	50
Brom-aspergillic Acid	1:1,600	25
Fumigacin	1:400	34
Gliotoxin	1:20,000	93

than is the motility, since satisfactory clinical results have been obtained with much lower blood levels of penicillin than indicated in Table III. It is, however, not unlikely that antibiotics other than penicillin might be found useful in spirochetal infections, provided the compounds used are of sufficiently low toxicity.

In this connection, it is of interest to note that Dunham and Rake (1945) found that purified penicillin was much less effective in inhibiting the *in vitro* motility of *Treponema pallidum* than partly purified preparations. Whether this difference in effectiveness holds true in the inhibition of the *in vivo* activity of these organisms is not known.

Finally, reference to Figure 1 discloses the fact that large gaps exist in our knowledge of the bacteriostatic effect of antibiotics upon various bacteria. A good deal of work yet remains before this aspect of antibiotic investigation can be considered complete.

METHODS OF ASSAY

Penicillin

Nearly all commonly used methods for the determination of penicillin, as well as of other antibiotics, are based upon microbiological principles. They depend upon the more or less quantitatively measured inhibitory action of the antibiotic upon bacterial growth, or, more rarely, upon some property of the organism which in turn is a function of growth (e.g., hemolysin production). A rough measure of penicillin concentration can be had by measuring the dilution required just

to inhibit the growth of a test organism in either broth or agar. Inasmuch as the dilutions ordinarily are serial dilutions, a factor of error of at least $\pm 50\%$ prevails.

Faced with the problem of developing a rapid assay procedure which would yield more quantitative results, the Oxford group introduced the cup method of assay in 1941 (Abraham, Chain, et al.). Hollow cylinders, which may be glass, metal or porcelain, are placed on an agar plate inoculated with a suitable microorganism (usually *Straphylococcus aureus*) so as to form a liquid-tight seal between the agar and the cylinder. The solution to be tested is placed in the "cup," and after incubation it is found that a circular area in which no growth has occurred surrounds the cylinder. The diameter of the zone of inhibition is proportional to the concentration of penicillin. Variations in diffusion rate, rate of growth, etc., are minimized by running a series of solutions of known concentrations on the same plate with the unknown.

The cup method of assay is convenient and sufficiently reliable ($\pm 20\%$) for many purposes. Only small amounts of solutions are required, and the sterility precautions required are minimal. Accurate measurement of zone diameters is, however, somewhat difficult, and the method is unsuited to the measurement of very low concentrations of penicillin. The latter difficulty has been partly overcome by a procedure which involves the addition of subinhibitory concentrations of a detergent to the agar used for seeding (T'ung, 1945). Synergism of penicillin and detergent effects considerable enlargement of the zone of inhibition.

A turbidimetric method for the determination of penicillin was first introduced by Foster (1942). Various concentrations of the unknown solution are prepared in broth, and the tubes are inoculated with a culture of a sensitive microorganism. After a suitable period of incubation, the extent of growth is measured photometrically. The growth is found to be inversely proportional to the penicillin concentration. The concentration of the unknown is found by comparison with a similarly treated series of tubes containing known amounts of penicillin.

Modifications of the turbidimetric assay have been developed by McMahon (1944), Joslyn (1944), and by Lee, Foley, Epstein and Wallace (1944).

The turbidimetric procedure, although somewhat less convenient than the cup method, is rapid, sensitive, and relatively accurate. In the author's laboratory an incubation period of 5–6 hours is used and concentrations of penicillin of 0.005 Oxford units per ml. can readily be measured. With some care, replicate determinations usually agree within 6–10%.

In an excellent summary of assay methods for penicillin, Foster and Woodruff (1943) described attempts to develop methods of analysis based upon principles other than those described above. The inhibition of the reduction of methylene blue by a penicillin-sensitive organism could not be used. Penicillin inhibits the luminescence of *Photobacterium fischeri*, but apparently large concentrations are required. Direct microscopic observation of the cessation of motility was found to be impractical, and alkali titration of the lactic acid produced by lactic acid bacteria was found to be inferior to photometric estimation of growth.

A chemical method for the determination of penicillin has been described by Murtaugh and Levy (1945). This method is based upon the observation that inactivation of penicillin (as by penicillinase) leads to the formation of another acid group. Penicillin solutions containing from 250 to 500 units per ml. can accurately be analyzed by alkali titration of the acid formed after treatment with penicillinase. The high concentrations required limit the application of this procedure, but this disadvantage is partly offset by the high accuracy obtainable (±2%).

An early report that the enzymatic activity of urease is inhibited by penicillin (Turner, Heath and Magasanik, 1943) led to the suggestion that this property could be utilized as a measure of penicillin concentration. Subsequent investigation revealed that the inhibition of urease is apparently a function of an impurity, since crystalline penicillin was inactive (Scudi and Jelnick, 1944).

One of the most important applications of penicillin assay procedures is the determination of blood levels following administration by various routes. At the present time no completely satisfactory method for the quantitative determination of penicillin in serum, plasma, or whole blood is available. The methods in use have been reviewed by Frieden and Frazier (1945). Briefly, there may be mentioned the dilution procedures of Rammelkamp (1942), of Fleming (1945), and of Kirby and Rantz (1944). An ingenious modification of the plate assay of penicillin has been applied to serum by Cooke (1945). The low serum levels of penicillin ordinarily found ordinarily preclude the routine use of the cup assay; Woolley and Schmidt (1945) have, however, suggested a modified cup assay applicable to blood level studies.

An accurate turbidimetric method for the estimation of penicillin in serum has been described by Kirby and Rantz (1944). The relatively large amounts of serum required seriously limits the routine application of this procedure.

Other Antibiotics

The general principles of microbiological assay described for penicillin can be applied in the determination of other antibiotics. A suitable test organism must be selected, chosen with particular regard to sensitivity and growth characteristics. Thus, *Bacillus subtilis* has been found to be a suitable organism for use in the cup assay of streptothricin (Foster and Woodruff, 1943). In addition, some of the other antibiotics possess specific properties which permit their assay by other than microbiological means. The hemolytic activity of tyrocidine has been made the basis of an accurate colorimetric analysis (Dimick, 1943).

In his original studies on lysozyme, Fleming compared the potencies of various preparations by measuring the maximum dilution capable of lysing heat—or phenol-killed cultures of *Micrococcus lysodeikticus* in a short time. Many subsequent investigators have used this method. Lysozyme assays were placed on a more quantitative basis by Boasson (1938) who

used the photometrically determined rate of lysis of heat-killed *M. lysodeikticus* as a measure of lysozyme activity.

The Mechanism of Antibiotic Action

Perhaps the most important current problem dealing with antibiotics is the elucidation of the mechanism whereby their antibacterial action is effected. The considerable volume of research connected with this subject which has so far been reported has not yet furnished sufficient information to enable the formulation of a rational hypothesis, although a good deal has been accomplished toward defining the conditions under which maximum antibacterial effect is achieved. Present-day knowledge of the chemical processes involved in bacterial nutrition and reproduction is very incomplete. This deficiency must be corrected before any considerable progress can be made toward a sound knowledge of the functions of compounds which interfere with these phenomena.

Mention has already been made of the mode of action of two of the substances with which this review is concerned. It appears to be well established that the lytic action of lysozyme arises from its catalysis of a hydrolytic reaction, converting a membrane polysaccharide into simpler, soluble compounds. The bacteriostatic action of notatin is reasonably attributed to the presence of hydrogen peroxide formed as a by-product of glucose oxidation.

Investigation of the mechanism of action of antibiotics other than lysozyme and notatin has been confined almost exclusively to gramicidin, tyrocidine, and penicillin. Despite considerable chemical similarity between gramicidin and tyrocidine, their effects on bacteria are apparently fundamentally different, and the available data indicate that penicillin functions in still another manner. It will be most convenient, therefore, to consider these three substances separately in some detail.

Gramicidin

Hotchkiss (1944) has ably reviewed the available information relating to the mechanism of gramicidin and tyrocidine action. While it is possible that the d-amino acid content of

both these compounds may confer toxic properties, or that the peptide of abnormal configuration may compete with some normal nutrient, he considers it more likely that the unnatural configuration confers some measure of biological indestructability upon a compound which is bacteriostatic for other reasons. There is some evidence that gramicidin, and particularly tyrocidine, bear some relation to the bacteriostatic detergents, since both these compounds exhibit surface tension depressant action at very low concentrations. These effects may be of some importance. It is likely, however, that other effects are more important.

A striking property of gramicidin is its effect upon the rate of respiration of susceptible cells. In concentrations approximating those at which its bacteriostatic action is evident, gramicidin markedly increases the oxygen uptake of suspensions of staphylococci, but has no effect upon organisms resistant to its bacteriostatic action. The stimulation persists for some time, following which the respiration gradually drops to zero. The stimulation of respiration applies only to suspensions in which glucose is the substrate, and under these conditions the respiratory quotient is unaffected. If lactate is present, there is no effect upon either the rate of oxygen uptake or the respiratory quotient. If glycerol is being oxidized, however, the rate of respiration is sharply inhibited, and at the same time the R.Q., which in the absence of gramicidin is below the theoretical for glycerol, closely approximates this figure. Hotchkiss interprets these results as indicating that in the absence of gramicidin some oxidation product of glycerol (not acetic acid) accumulates which cannot do so in its presence. Finally, experiments show that the removal of phosphate from the medium by washed staphylococci respiring in glucose is completely inhibited by gramicidin.

If it is assumed that, in normal respiratory activity, part of the glucose used is diverted from the main path of oxidation, the stimulation of oxygen utilization by gramicidin might be the result of an interference with this side reaction. It is suggested that the side reaction is the storage of a polysaccharide, the synthesis of which may be accomplished through

a phosphorylated glucose intermediate. Interference with the synthesis of this polysaccharide might very plausibly affect bacterial growth and especially reproduction. At any rate the action of gramicidin is fairly general; the effect of gramicidin upon the respiration of bacteria has its parallel in effects upon spermatozoa, muscle cells, and erythrocytes. The selective action of gramicidin may have its origin in the presence of inhibitors (as phospholipids) in insusceptible cells.

Tyrocidine

In contrast to gramicidin, tyrocidine depresses the respiratory activity of susceptible bacteria. A more striking effect is that tyrocidine causes the release of soluble cellular substances. When tyrocidine is rendered neutral, as by acetylation, its bactericidal activity is eliminated. The "leaching" effect of tyrocidine can be duplicated with other surface active agents. Hotchkiss concludes that tyrocidine is a bactericidal detergent ". . . .unique only in its natural origin and complexity of chemical composition."

Penicillin

Information pertinent to the mechanism of action of penicillin can be classified as follows:

1. Data relating to differences in sensitivity of different species and different strains of the same species.
2. Evidence for morphological changes induced in bacteria exposed to penicillin action.
3. The effect of penicillin in relation to conditions of bacterial growth.
4. The effect of inhibitors and synergic agents.

Antibiotics are sometimes roughly classified according to their comparative effects upon gram-positive and gram-negative bacteria. As indicated in the section on biological activity, this classification is relative, and may sometimes be misleading. Nevertheless, the difference is in many cases so striking as to be worth consideration, since it is possible that a knowledge of the essential differences between gram-positive and gram-negative forms may provide a valuable clue as to the mechanisms involved. Unfortunately, little is known about

the chemistry of these differences. Henry and Stacey (1943) studied the chemistry of some bacteria in relation to their staining characteristics. They concluded that the essential difference between gram-positive and gram-negative forms was the presence of a magnesium salt of a ribonucleic acid in the surface membrane of the former. Gram-positive organisms may be converted into gram-negative ones by altering the growth conditions in such a way as to prevent the formation of the magnesium compound. It has been shown (Shwartzman, 1945A) that the refractoriness to penicillin of some gram-negative bacteria *(E. Coli*, some *Salmonella)* is partly extrinsic in nature, since in very rich media the sensitivity of these organisms is less than under conditions such that the sole source of nitrogen is ammonia. Shwartzman later (1945B) showed that certain amino acids, notably methionine, threonine, and methionine sulfoxide considerably enhanced the effect of penicillin upon ordinarily refractory gram-negative species.

Another important distinction between gram-positive and gram-negative bacteria appears to be related to their comparative acid or basic nature (Dubos (1945). The "isoelectric points" (the pH at which acid and basic dyes are equally retained) of gram-positive species appear to cluster about pH 2.0, whereas those of gram-negative organisms group themselves in the neighborhood of 5.0. Some bacteria which commonly show variable response to the gram stain range in between the two groups. That this difference is a reflection of some fundamental constitutional difference between the two types is unquestioned, but its significance with respect to penicillin sensitivity remains obscure.

There is no apparent relationship between resistance to penicillin and the production of bacterial penicillinase (Woodruff and Foster, 1945). Evidence has been obtained for the production of penicillinase by sensitive forms, and some quite insensitive bacteria do not appear to produce the enzyme (Watson, 1944; Bondi and Dietz, 1944).

As has already been noted, naturally-occurring strains of various bacteria may sometimes display wide variations in

penicillin sensitivity. This property has not yet been correlated with any other characteristic property, with the possible exception of that of virulence.

In one of the early reports dealing with the biological properties of penicillin, Abraham and his co-workers (1941) stated that by serial passage of staphylococci upon penicillin-containing media a derived strain of greatly increased resistance could be isolated. It was found that the adapted strain was slower-growing as compared to the original culture, and that the rate of liquefaction of gelatin, coagulation of milk, etc., was considerably depressed. Since penicillin in contact with the resistant strain could be recovered with undiminished activity, it was apparent that the resistance was not due to the elaboration of a penicillin-destroying substance.

These observations have been abundantly confirmed. McKee and Houck (1943) obtained similar results with staphylococci, streptococci, and pneumococci, although the two latter species did not develop as strikingly high resistance as did the former. Small colony variants (G-forms) of *Staphylococcus albus*, sometimes observed in studies of the effect of penicillin, frequently proved to be exceptionally resistant (Schnitzer, Camagni, and Buck, 1943). Rammelkamp and Maxon (1942) have also observed the develpoment of resistance in staphylococci. Development of resistance by *in vivo* exposure to penicillin has been demonstrated for pneumococci (Schmidt and Sesler, 1943).

There is some evidence that strains of *Treponema pallidum* isolated from rabbits receiving subcurative doses of penicillin are less sensitive to penicillin than was the original strain (Dunham, Hamre, McKee, and Rake, 1944).

In contrast to the development of resistance to penicillin by staphylococci, streptococci, and pneumococci, there is no evidence that penicillin-resistant forms of *Gonococcus* are to be found in nature. In a study of 203 strains of gonococci isolated from clinical sources, Lankford (1945) found the resistance to penicillin confined to the limits of 0.0025–0.02 units per ml. Likewise, no increase in tolerance could be observed during the treatment with penicillin, although some of the

cases studied showed evidence of (somewhat delayed) relapse of infection.

A comprehensive summary of the available clinical and experimental data relating to the possibility of the induction or the acquisition of resistance to penicillin by *N. Gonorrhoeae* and *T. Pallidum* has been published by Frazier (1944).

Following 15 passages in penicillin-containing media, T'ung (1945) found only slight changes in the resistance of *Hemophilus ducreyi* to penicillin.

Careful studies on the development of penicillin resistance have been published by Demerec (1945) and by Todd, Turner, and Drew (1945). Starting with an initially sensitive strain of *Staphylococcus aureus*, Demerec found no change in the number of surviving organisms when a broth inoculum was plated onto agar containing from 0.0 to 0.012 units of penicillin per ml. At higher concentrations there was a progressive decrease in the number of surviving colonies until only one in one million survived at 0.05 units per ml. From the few survivors at 0.064 units per ml., strains resisting a concentration of 0.15 units per ml. could be isolated; from survivors at 0.125 units per ml. there were individuals resisting 0.25 units per ml. Further progressive resistance developed until a strain unaffected by 250 units per ml. was isolated. This represents an increase in tolerance of about 5000 fold.

Demerec found (1) that the resistance to penicillin was inherited (20 serial passages through broth containing no penicillin did not affect the resistance); (2) that it was not an acquired characteristic, dependent upon cell-antibiotic contact; and (3) that the evidence favors the acceptance of the theory that resistance occurs as a result of mutation, the origin of which is independent of penicillin treatment. Serial passage in agar containing increasing concentrations of penicillin thus functions as a selective mechanism, "screening out" the less resistant organisms.

Somewhat different results were obtained by Todd, Turner, and Drew. Using serial passage in broth containing penicillin, they were able to increase the resistance of the organism *(Staphylococcus)* some 3,000 times after 25 transfers. How-

ever, upon continued transfer in broth containing no penicillin, the resistance decreased rapily until reversion to the original sensitivity was nearly complete. It is difficult to tell whether the differences observed in inheritance of penicillin fastness by these two groups of workers is due merely to the different techniques (agar vs. broth) used, or represents some more profound variation. Rake and his associates (1944) have found that pneumococcal resistance to penicillin is retained upon subcultivation in the absence of penicillin.

The development of resistance of various microorganisms to penicillin has no apparent effect upon their sensitivity to the sulfonamides. Nor do penicillin resistant strains appear to be less susceptible to the antibiotics gramicidin, gliotoxin, or aspergillic acid (McKee and Houck, 1943).

The phenomenon of induced resistance apparently extends to antibiotics other than penicillin. Phillips and Barnes (1942) were able to increase the resistance of staphylococci to gramicidin to approximately 100 times the original tolerance. The development of resistance to tyrothricin has been reported by Rammelkamp (1942). Miller, Abrams, Dorfman, and Klein (1942) state that strains of *Escherichia coli* and *Eberthella typosa* originally sensitive to the bactericidal action of protomine could develop resistance to this antibacterial agent.

Along with the development of penicillin resistance in pathogenic organisms there is, in some instances at least, an associated decrease in virulence. This clinically fortunate circumstance was apparently first observed by McKee and Houck (1943). Staphylococci, streptococci, and type III pneumococci showed sharp decrease in virulence while increasing their sensitivity to penicillin from 30 to 4,000 fold. Pneumococci of types I and II were apparently unaffected. Both resistance and a virulence were maintained during serial passage through mice, transfers in broth, and storage.

Similar results were obtained by Rake, McKee, Hamre, and Houck (1944). Spink, Ferris, and Vivino (1944) showed that staphylococci trained to grow in the presence of penicillin were more susceptible to the bactericidal action of whole blood than were the original cultures.

The phenomenon of decreased virulence of penicillin resistant forms is in striking contrast to the situation with respect to the sulfonamides. Development of sulfonamide-resistant bacteria is generally unaccompanied by any change in virulence. The lack of sufficient data prohibits an extended discussion of the possible relationship of decreased virulence to the mechanism of penicillin action. As has been recently pointed out by Dubos (1945), ". . . . the term virulence does not refer to one specific attribute of the infective microorganisms, but expresses the ability of a given agent to establish under well-defined conditions a pathological state in a given host. . . . It is necessary to reiterate . . . that like the other characters of bacteria, the different components of virulence, such as communicability, resistance to the normal and immune defense mechanisms of the host, invasiveness, production of poisonous substances, etc., may vary independently of each other." The results obtained by Spink, Ferris, and Vivino are perhaps an indication that the alteration in the internal biochemical environment of a microorganism which results in substitution for or elimination of a penicillin sensitive reaction(s) simultaneously alters the ability of the organism successfully to resist the bactericidal action of the blood. In any case, there is little doubt that penicillin- and sulfonamide-tolerance are essentially unrelated phenomena.

Early studies on bacterial antagonists showed that anthrax bacilli subjected to the action of pyocyanase exhibited typical morphological changes (Emmerich and Saida, 1900). Similar effects were noted by Borodulina (1935), who showed that the antagonists formed by actinomycetes delayed cell division and produced elongation in *Bacillus mycoides*. Gardner (1940) was apparently the first to show that penicillin induced similar changes in microorganisms. General enlargement of *Clostridium welchii* in the presence of low concentrations of penicillin was followed by lengthening to ten times the normal cell length. The effect upon various species was found to be proportional to their penicillin sensitivity; highly resistant forms remained unaffected.

Smith and Hay (1942) demonstrated that like changes occurred in staphylococci. Swelling of inhibited cocci was followed by partial lysis. Detailed examination of individual organisms revealed a clear zone across the center of the cell, and it was suggested that perhaps it was the last step in mitosis which was affected by penicillin.

Similar effects have been shown for *Bacillus subtilis* by Foster and Woodruff (quoted by Waksman, 1945) and for *Hemophilus ducreyi* by T'ung. It appears that penicillin may exert analogous effects upon *Treponema pallidum*, at least *in vivo* (Frazier and Frieden, 1945).

In their early observations on penicillin Abraham and his associates (1941) found that even high concentrations of penicillin were not bactericidal, since large numbers of living bacteria were isolated from cultures exposed overnight to penicillin. These observations were extended by Hobby and coworkers (1942) who showed that penicillin was without effect at low temperatures, but exerted a killing effect under conditions favorable for growth. In such cases, the rate of killing varies logarithmically with the number of surviving organisms. Within limits, the bactericidal effect is proportional to the concentration of penicillin. Later studies revealed that the addition of substances which stimulated growth (paraamino benzoic acid, serum, glucose) increased the rate of killing (Hobby and Dawson, 1944).

That the effect of penicillin upon meningococci is greater under conditions favoring rapid multiplication was reported by Miller and Foster (1944). Lee, Foley, and Epstein (1944) showed that while low concentrations of penicillin were effective only against young cultures of staphylococci, very high concentrations were bactericidal for all forms, regardless of age. The logarithmic bactericidal effect described by Hobby was confirmed.

The effects of the factors of concentration, temperature, pH, and reproductive activity upon penicillin action was studied

by Garrod (1945), using the Oxford H strain of *Staphylococcus aureus*. The rate of killing appeared to be *inversely* proportional to penicillin concentration when commercial preparations of the drug were used, but when nearly pure samples were employed the rate was nearly independent of the concentration. This anomalous effect was attributed by Garrod to the presence of "inhibitors" in the preparations of lesser purity. (It should be noted that the concentration range employed by Garrod, 1.0 to 1,000 units per ml., is much higher than studied by others; this probably accounts for the fact that the bactericidal effect was found to be independent of concentration.)

Earlier reports that penicillin is effective only against dividing bacteria was based in part upon evidence that penicillin is inactive at lower temperatures. Garrod's results indicate a measurable activity at 4° (50% of the cell are killed in two days), and an increased bactericidal rate up to 42°. The increase in activity at 42° as compared with 37° is significant, since active growth of staphylococci ceases at the higher temperature. This fact emphasizes the similarity of penicillin to the ordinary disinfectants.

The rate of killing by penicillin increases with increase of pH in the range 5.0–7.5. Since maximum multiplication occurs near neutrality, these results may be suggestive of the primary effectiveness of penicillin during the division process. However, possible differences in the action of the different ionic forms of penicillin may be a factor here.

With respect to the conditions of nutrition and age of the test culture, Garrod was able to show that penicillin was slowly bactericidal in broth diluted sufficiently to prevent growth without affecting viability. It was also found that the effect of penicillin was substantially independent of the age of the culture; the rates of killing observed for 5-hour and 9-day cultures were only slightly different when the two were tested under nearly identical conditions. Again, a parallelism of effects between penicillin and the ordinary disinfectants (e.g., phenol) is to be noted.

In the presence of proflavine and sulfathiazole, Garrod found the penicillin was measurably less effective than in the absence of these bacteriostatic agents.

Another very careful study of the effect of penicillin upon growing bacterial cultures is that of Chain and Duthie (1945). It was found that penicillin was without effect upon the oxygen uptake of "resting" staphylococci (i.e., suspensions in which active growth no longer occurred). When the conditions were favorable to growth, however, a pronounced effect is observed. If low penicillin concentrations are used (0.04–0.1 units per ml.) an initial rise in oxygen consumption is followed by a decrease to a value well below the resting level. Still higher penicillin concentrations reduce the oxygen uptake almost immediately. If the effect upon growing cultures is followed turbidimetrically, an initial rise in turbidity is followed by a slow decrease; since cell counts failed to show an increase in the number of cells, the initial rise is attributed to increased size of individual organisms.

If penicillin is added to growing cultures during the logarithmic growth phase there is an immediate drop in oxygen consumption. This contrasts strongly with the initial rise which follows earlier addition (during the lag phase), and is taken to indicate that penicillin does not affect the increased metabolic activity which occurs immediately prior to the start of rapid multiplication.

Finally, the simultaneous addition of penicillin and a purely "bacteriostatic" agent (such as 'helvoic acid') to cultures in the lag phase does not decrease the oxygen uptake, although the increase in oxygen uptake which occurred in untreated controls was inhibited.

In contrast to the results obtained by Garrod with sulfathiazole, Chain and Duthie found that sulfamezathine synergized the bactericidal and bacteriolytic effects of penicillin. However, the differences in the two sets of experimental results may be due to concentration differences. Garrod used 0.2% sulfathiazole, a concentration high enough to completely inhibit growth, whereas the concentration of sufamezathine

employed by Chain and Duthie (0.05%) was low enough to permit the organisms to go through several cell divisions.

The data of Garrod and of Chain and Duthie agree in demonstrating that there is no evidence that penicillin exerts any but a purely bactericidal action. The fact that Garrod found little difference in the response to penicillin by young and old cultures, whereas Chain and Duthie found significant differences in effect upon oxygen uptake, may plausibly be ascribed to the fact that the former used higher concentrations of penicillin; part of the difference may also be due to technical factors. It is apparent that at low concentrations penicillin exerts its bactericidal effect only when a specific set of growth conditions prevail, but that higher concentrations are lethal in almost any circumstances.

The lytic effect of penicillin upon various bacteria has been investigated by Knox (1945), using both liquid and solid media. Lysis of staphylococci, pneumococci, and meningococci by penicillin was demonstrated, but streptococci were apparently resistant to the lytic action. With a rapidly growing staphylococcus measurable zones of inhibition around cups containing penicillin could be observed after five hours. These zones enlarged upon further incubation, indicating that colonies which had been visible earlier had been lysed. When penicillin was added at intervals to inoculated plates, the largest zones of inhibition were observed on plates to which penicillin had been added earliest. In agreement with other work, this fact implies that penicillin is most effective against young cultures. In broth, penicillin is lytic only if added before the end of the period of rapid growth.

The observations on the lytic effect of penicillin were extended to other types of pneumococci, clostridia, and various staphylococci and streptococci by Todd (1945A, B). The results obtained were essentially in agreement with those of Knox. It was concluded that the rate of lysis of bacteria exposed to the action of penicillin was dependent upon the rate of autolysin production. Thus penicillin, *per se*, appears to possess no lytic power, but by its killing action renders the cell more susceptible to natural autolytic processes.

In 1940, Abraham and Chain found that the apparent resistance of *Escherichia coli* to penicillin was at least partly due to the elaboration by this bacterium of a substance capable of destroying penicillin. This substance could be extracted from a crushed suspension of *E. coli;* it was found to be non-dialyzable, inactivated at 90°, and exhibited maximal activity at pH 8–9. These characteristics, together with the fact that the inhibitor can itself be inactivated by a proteolytic enzyme (papain), indicated that the inhibitory factor was enzymatic in nature. The enzyme was found to be formed intracellurally by a number of penicillin-resistant species, and it was also observed that at least one penicillin-sensitive organism *(Micrococcus lysodeikticus)* could produce it.

Later, Harper (1943) showed that the treatment of wounds with penicillin might be complicated by the presence therein of contaminating bacteria capable of producing the inhibitor. The term "penicillinase" was first introduced by Harper.

Duthie (1944) found that organisms of the *Bacillus subtilis* group produced fairly large quantities of penicillinase if penicillin was added late in the growth phase. A wider survey of bacteria for the production of the enzyme was made by Bondi and Dietz (1944). They found that coliform bacilli, many aerobic spore-formers, and certain shigellae showed evidence of its production.

More complete studies on penicillinase were reported by Woodruff and Foster (1944, 1945). Besides examining many species of bacteria for penicillinase production, they studied some of the factors involved in its action. The enzyme is active at pH 3–11, with maximal activity at pH 6.5–8.0. Its temperature optimum is 40°, and thermal inactivation occurs above 50°. Penicillinase is inactivated by sodium azide, iodoacetate, and ferrous chloride. Inactive penicillinase can sometimes be reactivated by sulfhydryl compounds (thioglycollate).

The exact effect of penicillinase upon penicillin is unknown. The fact that another acid group is formed following penicillin inactivation (Woodruff and Foster, 1945) suggests that

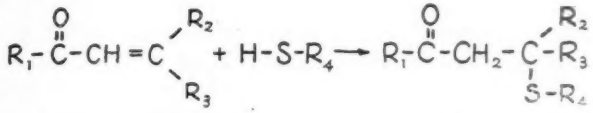
the essential reaction is one in which a lactone or lactam ring is opened; this would attribute to penicillinase some of the properties of a lipase or peptidase.

Penicillinase is not the only enzyme known to be capable of inactivating penicillin. Lawrence (1943) found that complete inactivation of penicillin in a short time was effected by the enzymes clarse and takadiastase. Later reports, however, attributed the activity of these preparations to the presence of bacterial contaminants. Lawrence later (1945) extended his studies to include other enzymes and found that papain, diastase, and amylase were also active.

The most intriguing studies on the inactivation of antibiotics, at least from the standpoint of the mechanism of action, are probably those dealing with the effects of certain specific chemical substances. Woodruff and Foster (1944), in their studies on penicillinase, mentioned that cysteine inactivated penicillin. Previously, however, Atkinson and Stanley (1943) had found that naturally-occurring suppressors of penicidin were apparently sulfhydryl compounds. Chow and McKee (1945) studied the effect of cysteine more carefully, and found that the inactivation was due to a slow reaction between cysteine and penicillin. The rate of the reaction was proportional to pH in the range 5.3–7.6. The inactivating effect of thioglycollate was evident only at concentrations much higher than those required for cysteine inactivation. Cystine, methionine, glycine and serine were without effect.

Inactivation by cysteine is not confined to penicillin. Cavallito and Bailey (1944) found that cysteine, as well as its esters, destroyed the antibacterial properties of citrinin, pyocyanine, gliotoxin, clavacin, and the antibiotic principles of a number of plants. The thio ethers of cysteine failed to reduce the activity of any of these compounds. Geiger and Conn (1945) tested a number of reducing agents, including cysteine, for inactivation of clavacin, penicillic acid, and a number of other α,β-unsaturated ketones which were shown to possess antibacterial activity. It was found that the antibiotic activity was abolished by cysteine for those compounds for which an

in vitro reaction with cysteine is possible. They formulate the reaction as:



Clavacin and penicillic acid are both sensitive to cysteine. Thioglycollate was found to be as effective as cysteine. It was suggested that the antibiotic activity of these compounds results from a reaction which involves the reactive sulfhydryl groups within the bacterial cell.

Colwell and McCall (1945) showed that the antibacterial action of 2-methyl, 1,4 naphthoquinone, of 3-chloro, 2-methyl, 1,4 naphthoquinone, and of 2, 6-dimethyl, 1,4 naphthoquinone could be suppressed by either thioglycollate or cysteine. These compounds are known to react with -SH compounds *in vitro*. On the other hand, 2-methyl, 3-methoxy, 1,4 naphthoquinone, highly active as an antibacterial, was unaffected by cysteine, and no *in vitro* reaction could be demonstrated.

More recently, it has been shown (Denkelwater, Cook, and Tishler, 1945) that cysteine at relatively high concentrations inactivates streptomycin, but has no apparent effect upon streptothricin. Inactivation of streptomycin by cysteine is reversed by iodine. 2-Amino-ethanol has an effect similar to that of cysteine, whereas thioglycollic acid is much less effective.

A few reports dealing with the synergic effect of both para-amino benzoic acid and the sulfonamides have already been mentioned. Although Hobby and Dawson (1944) could find no evidence that either para-amino benzoic acid, sulfadiazine, or sulfapyridine had any effect on penicillin action, Ungar (1943) found that both para-amino benzoic acid and sulfapyridine enhanced the effect of penicillin upon several bacterial species. Kirby (1944) also found evidence for synergism between penicillin and the sulfonamides. It is not unlikely that these conflicting results are due in part to variations in dosages, composition of the medium, etc. Studies in

chemically defined media will probably be necessary in order to resolve this interesting controversy.

It has been found in this laboratory that high concentrations of nicotinamide (1 mg. per ml.) significantly increases the sensitivity of *Staphylococcus aureus* to penicillin. Nicotinic acid is without effect. The high concentration of the amide required suggests the possibility of an impurity being the active agent.

Waksman (1945) has summarized the possible modes of action of antibacterial agents as follows:

1. The antibacterial may oxidize (reduce) a substance which should be reduced (oxidized) in the process of nutrition or growth.

2. The antibacterial may combine with a substrate or a nutritional factor, thereby rendering it inaccessible.

3. The antibacterial may compete for an enzyme required for an essential metabolic process.

4. The antibacterial may affect the surface tension of the cell, thus significantly altering the physiological state of the cell membrane.

Examples of some of the modes of action listed above are well known. Avidin functions as an antibacterial for those microorganisms which require biotin (mechanism 2). The well known interrelationship of the sulfonamides and paraamino benzoic acid is accepted as being evidence that the former function as enzyme inhibitors (mechanism 3), and the disinfectant action of some detergents, as well as the bactericidal action of tyrocidine can readily be explained on the basis of mechanism 4.

There is no *a priori* reason for assuming that the antibiotics must necessarily conform to one or another of the schemes listed above. It is, nevertheless, worth while to carefully examine our knowledge of antibiotic mechanisms in the light of these possibilities; such a consideration might reveal some preference for one of the four theories proposed.

There is no evidence that the bactericidal or bacteriostatic action of the antibiotics bears any simple relationship to their

oxidation reduction potentials. This may be merely a reflection of the lack of data; the experimental difficulties encountered in measuring such potentials under strictly reversible conditions are manifold. A possible fruitful field for investigation is indicated here.

In view of the fact that a large number of antibiotics are inactivated by sulfhydryl compounds, and that enzymatic processes in which sulfhydryl-disulfide equilibria are important are intimately concerned in cellular multiplication (Rapkine, 1938), several groups of workers have suggested that antibiotics may function by combining with the sulfhydryl groups of essential enzymes or essential sulfur-containing metabolites (Cavallito and Bailey, 1944; Geiger and Conn, 1945; Colwell and McCall, 1945). A similar mechanism was suggested earlier to account for the antitrypanocidal and antispirochetal action of mercury and arsenic compounds (Voegtlin, Dyer, and Leonard, 1923; Kolmer, Kast, and Rule, 1940). Some of the similarities and differences in the action of the arsenicals and the antibiotics have been discussed (Frazier and Frieden, 1945).

The ready reactivity of many antibiotics with sulfhydryl compounds *in vitro* affords little or no justification for the assumption that their *in vivo* action involves similar compounds. It is well known that penicillin, for example, can be recovered nearly quantitatively from suspensions of susceptible bacteria. Its effect must therefore be a reversible one, and if it acts upon one or another of the labile sulfhydryl groups within the bacterial cell, its inhibitory effect should be readily reversed by the addition of an appropriate sulfur-containing compound. True reversibility of antibiotic action effected by thiols (or any other compounds) has yet to be demonstrated. It is further evident that hypothetical effects upon sulfur-containing enzymes or substrates can at best be only part of the story. Ample authority has been cited to show that not all antibiotics are sensitive to the action of either cysteine or thioglycollate. Finally, the fact that development of resistance to the action of one antibiotic does not appear to

confer tolerance to another makes untenable the hypothesis that antibiotics affect the same phenomena.

No direct evidence is available regarding the possibility that penicillin acts by interfering with the utilization of an essential metabolite, somewhat as the utilization of para-amino benzoic acid is inhibited by various sulfonamides, but speculation as to the possible nature of the hypothetical metalobite is not unwarranted. It has been shown that the concentration of sulfonamide required for complete bacteriostasis depends in part upon the concentration of para-amino benzoic acid. Experiment shows that from 1 to 26,000 moles of sulfonamide may be required to block the utilization of one mole of para-amino benzoic acid, depending upon the conditions employed. The average ratio is probably not far from 1,000. Penicillin, as we have seen, is effective against some staphylococci at concentrations of 0.01 γ per ml. Assuming a molecular weight of 500, the effective concentration of penicillin may thus be as low as 2×10^{-8} M. The effective ratio of penicillin to the hypothetical intermediate with which it may be presumed to interfere is, of course, unknown, but a ratio of 100:1 may not be an unreasonable guess. It follows, therefore, that the hypothetical metabolite may be effective at concentrations below 10^{-10} M. This is far below the concentrations at which most growth factors, even those of the vitamin B group, are ordinarily effective. Knight (1937) found that the thiamine and nicotinic acid requirements of staphylococci were approximately 10^{-7} M and 10^{-5} M, respectively. The riboflavin requirements of *Sarcina lutea* (Sartory, Sartory, and Meyer, 1938) and of *Lactobacillus casei* (Williams and his associates, 1932) also appear to be in the neighborhood of 10^{-7} M. The pyridoxin requirement of *Staphylococcus albus* (Vilter and Spies, 1940 is about 10^{-6} M. *Lactobacillus casei* requires pantothenic acid in concentrations of 10^{-7} M for good growth (Williams, et al., 1942).

A different situation obtains with respect to the growth factors biotin and folic acid. Biotin is known to stimulate the growth of streptococci at 3×10^{-10} M (Hottle, Lampen, and

Pappenheimer, 1941), and is effective at even lower concentrations for various clostridia. Even quite impure preparations of folic acid are effective in stimulating the growth of streptococci at 2×10^{-9} M.

Experiments by the author have shown that folic acid has no effect on the penicillin inhibition of staphylococci even when the former is present in concentrations several thousand times the figure given above. Similar experiments (Lankford and Frieden, unpublished data) indicate that riboflavin (10 γ per ml.) likewise has no effect on penicillin inhibition of *Lactobacillus casei*. As noted above, nicotinamide has an enhancing rather than an antagonistic effect upon penicillin. Finally, no data are available relating to the effects of biotin, thiamin, or the other B vitamins.

The possibility of metabolic interference by penicillin remains as a hypothetical mode of action. Careful studies of the nutritional requirements of microorganisms, particularly in relation to penicillin sensitivity, will be required to verify or disprove this hypothesis. In the field of antibiotics, such studies are perhaps the most urgently needed investigations at the present time.

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REFERENCES

Abraham, E. P.: Some properties of egg-white lysozyme, Biochem. J., 33:622–630, 1939.

Abraham, E. P., Baker, W., Chain, E., Florey, H. W., Holiday, E. R., and Robinson, R: Nitrogenous character of penicillin, Nature, 149: 356, 1942.

Abraham, E. P., and Chain, E.: An enzyme from bacteria able to destroy penicillin, Nature, 146:837, 1940.

Abraham, E. P., and Chain, E.: Purification and some physical and chemical properties of penicillin, Brit. J. Ex. Path., 23:103–119, 1942.

Abraham, E. P., Chain, E., Baker, W., and Robinson, R.: Penicillamine, a characteristic degradation product of penicillin, Nature, 151:107, 1943.

Abraham, E. P., Chain, E., Fletcher, C. M., Gardner, A. D., Heatley, N. G., Jennings, M. A., and Florey, H. W.: Further observations on penicillin, Lancet, 2:177–189, 1941.

Alderton, G., Lewis, J. C., and Fevold, H. L.: The relationship of lysozyme, biotin, and avidin, Science, 101:151, 1945.

Alderton, G., Ward, W. H., and Fevold, H. L.: Isolation of lysozyme from egg-white, J. Biol. Chem., 157:43–57, 1945.

Alsberg, C. L., and Black, O. F.: Contribution to the study of maize deterioration; biochemical and toxocological investigations of *Penicillium puberulum* and *Penicillium stoloniferum*, U.S.D.A., Bur. of Plant Industry, Bull. 270, 1913.

Anslow, W. K., and Raistrick, H.: Studies in the biochemistry of microorganisms; fumigatin (3-hydroxy, 4-methoxy, 2,5-toluquinone) and spinulosin (3,6-dihydroxy, 4-methoxy, 2,5-toluquinone), metabolic products respectively of *A. fumigatus* Fresenius and *P. spinulosum* Thom, Biochem. J., 32:687–696, 1938.

Armstrong, W. D., Spink, W. W., and Kahnke, J.: Antibacterial effects of quinones, Proc. Soc. Exp. Biol. Med., 53:230–234, 1943.

Atkinson, N.: Antibacteria substances produced by moulds. I. Penicidin, a product of the growth of a *Penicillium*, Austra. J. Exp. Biol. Med. Sci., 20:287–288, 1942.

Atkinson, N.: Antibacterial substances produced by moulds. II. Antibacterial substances produced by some common penicillia, Austra. J. Exp. Biol. Med. Sci., 21:15–16, 1943.

Atkinson, N., and Stanley, N. F.: Antibacterial substances produced by moulds. V. Mechanism of action of some penicidin suppressors, Austra. J. Exp. Biol. Med. Sci., 21:249–253, 1943.

Barger, G., and Dorrer, O.: Chemical properties of puberulic acid, $C_8H_6O_6$, and a yellow acid, $C_8H_4O_6$, Biochem. J., 28:11–15, 1934.

Bergel, F., Morrison, A. L., Moss, A. R., Klein, R., Rinderknecht, H., and Ward, J. L.: An antibacterial substance from *Aspergillus clavatus* and *Penicillium claviforme* and its probable identity with patulin, Nature, 152:750, 1943.

Belozersky, A. N., and Passhina, T. S.: Chemistry of gramicidin S, Lancet, 2:716–717, 1944.

Birkinshaw, J. H., Oxford, A. E., and Raistrick, H.: Studies in the biochemistry of microorganisms; penicillic acid, a metabolic product of *Penicillium puberulum* Banier and *Penicillium cyclopium* Westling, Biochem. J., 30:394–411, 1936.

Birkinshaw, J. H., and Raistrick, H.: Studies in the biochemistry of microorganisms; puberulic acid, $C_8H_6O_6$ and an acid $C_8H_4O_6$, new products of the metabolism of glucose by *Penicillium puberulum* Bainier and *Penicillium aurantio-virens* Biourge, Biochem. J., 26:441–453, 1932.

Birkinshaw, J. H., and Raistick, H.: Notatin: an antibacterial glucose aerodehydrogenase from *P. notatum* Westling, J. Biol. Chem., 148: 459–460, 1943.

Birkinshaw, J. H., Raistrick, H., and Smith, G.: Studies in the biochemistry of microorganisms. 71. Fumaryl dl-alanine, a metabolic product of *Penicillium resticulosum*, Biochem. J., 36:829–835, 1942.

Boasson, E. H.: On the bacteriolysis by lysozyme, J. Immunol., 34:281–293, 1938.

Bondi, A., and Dietz, C. C.: Production of penicillinase by bacteria, Proc. Soc. Exp. Biol. Med., 56:132–134, 1944A.

Bondi, A., and Dietz, C. C.: Relationship of penicillinase to the action of penicillin, Proc. Soc. Exp. Biol. Med., 56:135–137, 1944B.

Borodulina, J. A.: Interrelationships of soil actinomyces and *B. mycoides*, Microbiologia (USSR), 4:561–586, 1935.

Bovarnick, M.: The formation of extracellular(—)-glutamic acid polypeptide by *Bacillus subtilis*, J. Bio. Chem., 145:415–424, 1942.

Bruce, W. F., Dutcher, J. D., Johnson, J. R., and Miller, L. L.: Gliotoxin, the antibiotic principle of *Gliocladium fimbriatum*. II. General chemical behavior and crystalline derivatives, J. Am. Chem. Soc., 66:614–616, 1944.

Bush, M. T., and Goth, A.: Flavicin; an antibacterial substance produced by an *Aspergillus flavus*, J. Pharm. and Exp. Ther., 78:164–169, 1943.

Catch, J. R., Cook, A. H., and Heilbron, I. M.: Purification and chemistry of penicillin, Nature, 150:633–634, 1942.

Cavallito, C. J., and Bailey, J. H.: Allicin, the antibacterial principle of *Allium sativum*. I. Isolation, physical properties, and antibacterial action, J. Am. Chem. Soc., 66:1950–1951, 1944A.

Cavallito, C. J., and Bailey, J. H.: Preliminary note on the inactivation of antibiotics, Science, 100:390, 1944B.

Cavallito, C. J., Bailey, J. H., and Buck, J. S.: The antibacterial principle of *Allium sativum*. III. Its precursor and 'essential oil of garlic,' J. Am. Chem. Soc., 67:1032–1033, 1945.

Cavallito, C. J., Bailey, J. H., and Kirchner, F. K.: The antibacterial principle of *Arctium minus*. I. Isolation, physical properties, and antibacterial action, J. Am. Chem. Soc., 67:948–950, 1945.

Cavallito, C. J., Buck, J. S., and Suter, C. M.: Allicin, the antibacterial principle of *Allium sativum*. II. Determination of the chemical structure, J. Am. Chem. Soc., 66:1952–1954, 1944.

Cavallito, C. J., Kirchner, F. K., Miller, F. C., Bailey, J. H., Klimek, J. W., Warner, W. F., Suter, C. M., and Tainter, M. L.: The benzyl ester of penicillin, Science, 102:150–151, 1945.

Chain, E., and Duthie, E. S.: Bactericidal and bacteriolytic action of penicillin on the staphylococcus, Lancet, 1:652–656, 1945.

Chain, E., Florey, H. W., and Jennings, M. A.: Identity of patulin and claviformin, Lancet, 1:112–114, 1944.

Chain, E., Florey, H. W., Jennings, M. A., and Callow, D.: An antibacterial substance produced by *Penicillium claviforme*, Brit. J. Exp. Path., 23:202–205, 1942.

Chain, E., Florey, H. W., Jennings, M. A., and Williams, T. I.: Helvolic acid, an antibiotic produced by *Aspergillus fumigatus*, mut. Helvola Yuill, Brit. J. Exp. Path., 24:108–119, 1943.

Chow, B. F., and McKee, C. M.: Inactivation of the antibiotic activity of penicillin by cysteine hydrochloride. I. Chemical aspects of inactivation, Proc. Soc. Exp. Biol. Med., 58:175–177, 1945.

Christensen, H. N.: Isolation of valylvaline from gramicidin hydrolyzates, J. Biol. Chem., 151:319–324, 1943.

Christensen, H. N., Edwards, R. R., and Piersma, H. D.: The composition of gramicidin and tyrocidine, J. Biol. Chem., 141:187–195, 1941.

Colwell, C. A., and McCall, M.: Studies on the mechanism of antibacterial action of 2-methyl, 1,4-naphthoquinone, Science, 101:592–593, 1945.

Cook, A. H., and Lacey, M. S.: An antibiotic from *Aspergillus parasiticus*, Nature, 153:460, 1944.

Cooke, J. V.: A simple clinical method for the assay of penicillin in body fluids and for testing of penicillin sensitivity of bacteria, J. Am. Med. Assoc., 127:445–449, 1945.

Coulthard, C. E., Michaelis, R., and Short, W. F.: British patent applica. No. 13242, 1941.

Coulthard, C. E., Short, W. F., Michaelis, R., Sykes, G., Skrimshire, G. E. H., Standfast, A. F. B., Birkinshaw, J. H., and Raistrick, H.: Notatin: an antibacterial glucose dehydrogenase from *P. notatum* Westling, Nature, 150:634, 1942.

Delbruck, M.: Bacterial viruses (bacteriophages), Adv. in Enzymol., 2: 1–32, 1942.

Demerec, M.: Production of *Staphylococcus* strains resistant to various concentrations of penicillin, Proc. Nat. Acad. Sci., 31:16–24, 1945.

Denkelwater, R. G., Cook, M. A., and Tishler, M.: The effect of cysteine on streptothricin and streptomycin, Science, 102:12, 1945.

D'Herrelle, F.: The Bacteriophage and its Clinical Applications, Springfield, 1930. C. C. Thomas.

Dimick, K. P.: A quantitative method for the estimation of tyrothricin, J. Biol. Chem., 149:387–393, 1943.

Dubos, R. J.: Bactericidal effect of an extract of a soil bacillus on Gram-positive cocci, Proc. Soc. Exp. Biol. Med., 40:311–312, 1939.

Dubos, R. J.: The Bacterial Cell, Cambridge, 1945. Harvard University Press.

Duffin, W. M., and Smith, S.: Penillic acid, an optically active acid from penicillin, Nature, 151:251, 1943.

Dunham, W. B., Hamre, D. M., McKee, C. M., and Rake, G.: Action of penicillin and other antibiotics on *Treponema pallidum*, Proc. Soc. Exp. Biol. Med., 55:158–160, 1944.

Dunham, W. B., and Rake, G.: The relative activity of partially purified penicillin and of crystalline penicillin G on *Treponema pallidum*, Am. J. Syph., Gon., and Ven. Dis., 29:214, 1945.

Dutcher, J. D., Johnson, J. R., and Bruce, W. F.: Gliotoxin, the antibiotic principle of *Gliocladium fimbriatum*. III. The structure of gliotoxin; degradation by hydroiodic acid, J. Am. Chem. Soc., 66: 617–621, 1944.

Dutcher, J. D., and Wintersteiner, O.: The structure of aspergillic acid, J. Biol. Chem., 155:359–360, 1944.

Duthie, E. S.: The production of penicillinase by organisms of the subtilis group, Brit. J. Exper. Path., 25:96–100, 1944.

Emmerich, R., and Löw, O.: Die kunstliche Darstellung der immunisierenden Substanzen (Nukleasen-Immunproteide) und ihre verwendung zur Therapie der infektionskrankheiten und zur Schutzimpfung an Stelle des Heilserums, Z. fur Hyg. und Infectionskr., 36:9, 1901.

Emmerich, R., and Saida, Dr.: Ueber die morphologischen Veränderungen der Milzbrandbacillen bei ihrer Auflösung durch Pyocyanase, Centralbl. f. Bakteriol., I, 27:776–787, 1900.

Fleming, A.: On a remarkable bacteriolytic element found in tissues and secretions, Proc. Roy. Soc. (London), 93B, 306–317, 1922.

Fleming, A.: On the antibacterial action of cultures of a *Penicillium*, with special reference to their use in the isolation of *B. influenzae*, Brit. J. Exp. Path, 10:226–236, 1929.

Fleming, A.: Micro-methods of estimating penicillin in serum and other body fluids, Am. J. Clin. Path., 15:1–6, 1945.

Florey, H.: The relative amounts of lysozyme present in the tissues of some mammals, Brit. J. Exp. Path., 11:251–261, 1930.

Florey, H. W., Heatley, N. G., Jennings, M. A., and Williams, T. I.: Penicillin-like antibiotics from various species of molds, Nature, 154: 268, 1944.

Florey, H. W., Jennings, M. A., and Philpot, F. J.: Claviformin from *Aspergillus giganteus Wehm*, Nature, 153:139, 1944.

Foster, J. W.: Quantitative estimation of penicillin, J. Biol. Chem., 144: 285–286, 1942.

Foster, J. W., and Karow, E. O.: Microbiological aspects of penicillin, VIII. Penicillin from different fungi, J. Bact., 49:19–28, 1945.

Foster, J. W., and Woodruff, H. B.: Quantitative estimation of streptothricin, J. Bact., 45:408–409, 1943A.

Foster, J. W., and Woodruff, H. B.: Microbiological aspects of penicillin. I. Methods of assay, J. Bact., 46:187–202, 1943B.

Frazier, C. N.: Microbial adaptation to penicillin, Texas Rep. Biol. Med., 2:385–396, 1944.

Frazier, C. N., and Frieden, E. H.: The mode of action of penicillin, with special reference to *Treponema pallidum*, J. Am. Med. Assoc. (in press).

Frieden, E. H.: The diffusion constant of penicillin, Science, 101:21, 1945.

Frieden, E. H., and Frazier, C. N.: Studies on penicillin in blood. I. The effect of serum and serum filtrates upon the growth of *Staphylococcus aureus*, J. Bact. (in press).

Gardner, A. D.: Morphological effects of penicillin on bacteria, Nature, 146:837–838, 1940.

Gardner, A. D., and Chain, E.: Proactinomycin: a bacteriostatic substance produced by a species of proactinomyces, Brit. J. Exp. Path., 23:123–127, 1942.

Garrod, L. P.: Action of penicillin on bacteria, Brit. Med. J., 1:107–110, 1945.

Gause, G. F., and Brazhnikova, M. G.: Gramicidin S—origin and mode of action, Lancet, 2:715–716, 1944.

Geiger, W. B., and Conn, J. E.: The mechanism of the antiobiotic action of clavacin and penicillic acid, J. Am. Chem. Soc., 67:112–116, 1945.

Geiger, W. B., Conn, J. E., and Waksman, S. A.: Chaetomin, a new antibiotic substance produced by *Chaetomium cochliodes*. II. Isolation and concentration, J. Bact., 48:531–536, 1944.

Gordon, A. H., Martin, A. J. P., and Synge, R. L. M.: The amino acid composition of gramicidin, Biochem. J., 37:86–92, 313–318, 1943.

Gratia, A., and Dath, S.: De l'action bacteriolytiques des streptothrix, Compt. rend. Soc. de biol., 91:1442–1443, 1924.

Green, D. E., and Pauli, R.: The antibacterial action of the xanthine oxidase system, Proc. Soc. Exp. Biol. Med., 54:148–150, 1943.

Harper, G. J.: Inhibition of penicillin in routine culture media, Lancet, 2:569–571, 1943.

Heatley, N. G.: An antibiotic from *Crepis tarazacifolia*, Brit. J. Exp. Path., 25:208–211, 1944.

Henry, H., and Stacey, M.: Histochemistry of the Gram-staining reaction for microorganisms, Nature, 151:671, 1943.

Hettche, H. O., and Weber, B.: Die Ursache der bakteriziden Wirkung von Mesentericus-filtraten, Arch. fur Hyg., 123:69–80, 1939.

Hobby, G. L., and Dawson, M. H.: Effect of rate of growth of bacteria upon the action of penicillin, Proc. Soc. Exp. Biol. Med., 56:181–184, 1944A.

Hobby, G. L., and Dawson, M. H.: Relation of penicillin to sulfonamide action, Proc. Soc. Exp. Biol. Med., 56:184–187, 1944B.

Hobby, G. L., Meyer, K., and Chaffee, E.: Observations on the mechanism of action of penicillin, Proc. Soc. Exp. Biol. Med., 50:281–285, 1942.

Hoogerheide, J. C.: An agent, isolated from a soil bacillus, which inhibits encapsulation of Friedländer's bacillus and is highly bactericidal for Gram-positive organisms, J. Frank. Inst., 677–680, 1940.

Hotchkiss, R. D.: The chemical nature of gramicidin and tyrocidine, J. Biol. Chem., 141:171–185, 1941.

Hotchkiss, R. D.: Gramicidin, tyrocidine, and tyrothricin, Adv. in Enzymol., 4:153–199, 1944.

Hottle, G. A., Lampen, J. O., and Pappenheimer, A. M., Jr.: Biotin as a growth factor for C-203S strain of hemolytic *Streptococcus*, Group A, J. Bio. Chem., 137:457–458, 1941.

Jansen, E. F., and Hirschmann, D. J.: Subtilin; an antibacterial product of *Bacillus subtilis;* culturing conditions and properties, Arch. Biochem., 4:297–309, 1944.

Johnson, J. R., Bruce, W. F., and Dutcher, J. D.: Gliotoxin, the antibiotic principle of *Gliocladium fimbriatum*. I. Production, physical and biological properties, J. Am. Chem. Soc., 65:2005–2009, 1943.

Joslyn, D. A.: Penicillin assay; outline of a four-hour turbidimetric method, Science, 99:21–22, 1944.

Karow, E. O., and Foster, J. W.: An antibiotic substance from species of *Gymnoascus* and *Penicillium*, Science, 99:265–266, 1944.

Kirby, W. M. M.: Bacteriostatic action of sulfonamide-penicillin and urea-penicillin mixtures *in vitro*, Proc. Soc. Exp. Biol. Med., 57:149–151, 1944.

Kirby, W. M. M., and Rantz, L. A.: Methods of measuring penicillin concentrations in body fluids, J. Bact., 48:603–608, 1944.

Knight, B. C. J. C.: The nutrition of *Staphylococcus aureus*. The activities of nicotinamide, aneurin (vitamin B_1) and related compounds, Biochem. J., 31:966–973, 1937.

Knox, R.: The effect of penicillin on cultures in liquid and solid media, Lancet, 1:559–561, 1945.

Kocholaty, W.: Microbial antagonisms and *Brucella abortus*, J. Bact., 44: 143, 1942.

Kocholaty, W.: Purification and properties of the second antibacterial substance produced by *Penicillum notatum*, Science, 97:186, 1943.

Kolmer, J. A., Kast, C. C., and Rule, A. M.: The spirocheticidal, trypanocidal, and mechanism of activity of organic arsenical compounds *in vitro* and *in vivo* in relation to therapeutic effectiveness, Am. J. Syph., Gon., & Ven. Dis., 24:201–222, 1940.

Krueger, A. P.: The nature of bacteriophage and its mode of action, Physiol. Rev., 16:129–172, 1936.

Krueger, A. P., and Scribner, E. J.: Bacteriophage therapy, J. Am. Med. Assoc., 116:2160–2167, 1941.

Lankford, C. E.: The *in vitro* tolerance of the gonococcus for penicillin, Am. J. Syph., Gon., & Ven. Dis., 29:56–63, 1945.

Laurence, W. L.: On the possible identity of avidin and egg-white lysozyme, Science, 99:392, 1944.

Lawrence, C. A.: Sterility test for penicillin, Science, 98:413–414, 1943.

Lawrence, C. A.: Effects of enzyme preparations upon penicillin. I. A. method for testing penicillin for sterility, J. Bact., 49:47–55, 1945.

Lee, S. W., Foley, E. J., and Epstein, J. A.: Mode of action of penicillin. I. Bacterial growth and penicillin activity. *Staphylococcus aureus* FDA, J. Bact., 48:393–399, 1944.

Lee, S. W., Foley, E. J., Epstein, J. A., and Wallace, J. H., Jr.: Improvements in the turbidimetric assay for penicillin, J. Biol. Chem., 152:485–486, 1944.

Lipmann, F., Hotchkiss, R. D., and Dubos, R. J.: The occurrence of d-amino acids in gramicidin and tryocidine, J. Biol. Chem., 141: 163–169, 1941.

Mattick, A. T. R., and Hirsch, A.: A powerful inhibitory substance produced by group N. streptococci, Nature, 154:551, 1944.

McDonald, E.: Further studies on the bactericidal agents obtained from soil bacilli, J. Frank. Ins., 229:805–815, 1940.

McIlwain, H.: Antibacterial action of two bacterial products of known structure, Nature, 148:628, 1941.

McKee, C. M., and Houck, C. L.: Induced resistance to penicillin of cultures of staphylococci, pneumococci, and streptococci, Proc. Soc. Exp. Biol. Med., 53:33–34, 1943.

McMahan, J. R.: An improved short time turbidimetric assay for penicillin, J. Biol. Chem., 153:249–258, 1944.

Menzel, A. E. O., Wintersteiner, O., and Hoogerheide, J. C.: The isolation of gliotoxin and fumigacin from culture filtrates of *Aspergillus fumigatus*, J. Biol. Chem., 152:419–429, 1944.

Meyer, K.: The relationship of lysozyme to avidin, Science, 99:391, 1944.

Meyer, K., Chaffee, E., Hobby, G. L., Dawson, M. H., Schwenk, E., and Fleischer, G.: On penicillin, Science, 96:20–21, 1942.

Meyer, K., Hobby, G. L., and Chaffee, E.: On esters of penicillin, Science, 97:205–206, 1943.

Meyer, K., Thompson, R., Palmer, J. W., and Khorazo, D.: The purification and properties of lysozyme, J. Biol. Chem., 113:303–309, 1936.

Miller, B. F., Abrams, R., Dorfman, A., and Klein, M.: Antibacterial properties of protamine and histone, Science, 96:428–430, 1942.

Miller, C. P., and Foster, A. Z.: Studies on the action of penicillin. III. Bactericidal action of penicillin on meningococcus *in vitro*, Proc. Soc. Exp. Biol. Med., 56:205–208, 1944.

Much, H.: Ueber den Bac. cytolyticus, München. Med. Wchnschr., 72: 374, 1925.

Much, H., and Sartorius, F.: Über die neuartige Lysine des Mycoides "Much," München. Med. Klinik, 20:345–348, 1924.

Murtaugh, J. J., and Levy, G. B.: Chemical method for the determination of penicillin, J. Am. Chem. Soc., 67:1042, 1945.

Nicolle, M.: Action du '*Bacillus subtilis*' sur diverses bacteries, Ann. Inst. Pasteur, 21:613–621, 1907.

Osborn, E. M.: On the occurrence of antibacterial substances in green plants, Brit. J. Exp. Path., 24:227–231, 1943.

Oxford, A. E.: Antibacterial substances from moulds. V. The bacteriostatic powers of the methyl ethers of fumigatin and spinulosin and other hydroxy-, methoxy-, and hydroxymethoxy- derivatives of toluquinone and benzoquinone, Chem. and Ind., 189–192, 1942.

Oxford, A. E.: Diplococcin, an antibacterial protein elaborated by certain milk streptococci, Biochem. J., 38:178–182, 1944.

Oxford, A. E., Raistrick, H., and Smith, G.: Antibacterial substances from moulds. II. Penicillic acid, a metabolic product of *P. puberulum* Banier and *P. cyclopium* Westling, Chem. and Ind., 22–24, 1942.

Oxford, A. E., Raistrick, H., and Smith, G.: Antibacterial substances from moulds. VI. Puberulic acid, $C_8H_4O_6$, and puberulonic acid, $C_8H_4O_6$, metabolic products of a number of species of *Penicillium*, Chem. and Ind., 485–487, 1942.

Peterson, W. H., McDaniel, L. E., and McCoy, E.: Biotin requirements of clostridia and assay of biological materials for biotin, J. Biol. Chem. 133:lxxv–lxxvi, 1940. (In soc. proc.)

Penau, M. H., Levaditi, C., and Hagemann, G.: Essais d'extraction d'une substance bactericide d'origine fungique, Bull. Soc. Chim. biol., 25: 406–410 (1943); Chem. Abstr., 38:5784, 1944.

Phillips, R. L., and Barnes, L. H.: Development of resistance in staphylococci to natural inhibitory substances (gramicidin), J. Frank. Inst., 233:396–401, 1942.

Philpot, F. J.: A penicillin-like substance from *Aspergillus giganteus* Wehm, Nature, 152:725, 1943.

Pratt, R., Daniels, T. C., Eiler, J. J., Gunnison, J. B., Kumler, W. D., Oneto, K. F., Strait, L. A., Spoehr, H. A., Hardin, G. J., Milner, H. W., Smith, J. H. C., and Strain, H. H.: Chlorellin, an antibacterial substance from *Chlorella,* Science, 99:351–352, 1944.

Puetzer, B., Neild, C. H., and Barry, R. H.: The synthesis of a clavacin isomer, Science, 101:307–308, 1945.

Raistrick, H., Birkinshaw, J. H., Michael, S. E., Bracken, A., Gye, W. E., and Hopkins, W. A.: Patulin in the common cold. Biochemical chemical, and biological properties, Lancet, 2:625–635, 1943.

Raistrick, H., and Smith, G.: Antibacterial substances from moulds. I. Citrinin, a metabolic product of *P. citrinum* Thom, Chem. and Ind., 828–830, 1941.

Rake, G., McKee, C. M., Hamre, D. M., and Houck, C. L.: Studies on penicillin. II. Observations on therapeutic activity and toxicity, J. Immunol., 48:271–289, 1944.

Rammelkamp, C. H.: Observations on resistance of *Staphylococcus aureus* to action of tyrothricin, Proc. Soc. Exp. Biol. Med., 49:346–350, 1942A.

Rammelkamp, C. H.: A method for determining the cencentration of penicillin in body fluids and exudates, Proc. Soc. Exp. Biol. Med., 51:95–97, 1942B.

Rammelkamp, C. H., and Maxon, T.: Resistance of *Staphylococcus aureus* to the action of penicillin, Proc. Soc. Exp. Biol. Med., 51:386–389, 1942.

Rapkine, L.: Sulfhydryl groups and enzymic oxidation-reduction, Biochem. J., 32:1729–1739, 1938.

Roberts, E. A. H.: The preparation and properties of purified egg-white lysozyme, Quart. J. Exp. Phyisol., 27:89–97, 1938.

Roberts, E. A. H., Maegraith, B. J., and Florey, H. W.: A comparison of lysozyme preparations from egg-white, cat, and human saliva, Quart. J. Exp. Physiol., 27:381–391, 1938.

Roberts, E. C., Cain, C. K., Muir, R. D., Reithel, F. J., Gaby, W. L., van Bruggen, J. T., Homan, D. M., Katzman, P. A., Jones, L. R., and Doisy, E. A.: Penicillin B, an antibacterial substance from *Penicillium notatum,* J. Biol. Chem., 147:47–58, 1943.

Rosenthal, L.: La lyse des *Bacilles diphtheriques* effectuee' par un *Streptothrix*, Compt. rend. Soc. de biol., 93:77–78, 1925.

Sanders, D., Weatherwax, P. W., and McClung, L. S.: Antibacterial substances from plants collected in Indiana, J. Bact., 49:206, 1945. (In Soc. Proc.)

Sartory, A., Sartory, R., and Meyer, J.: The influence of certain water soluble vitamins on the rate of growth of different coccoid bacteria, Compt. rend. Acad. d. sc., 206:1414–1416, 1938.

Schales, O.: Mode of action of some antibacterial mold products, Arch. Biochem., 2:487–489, 1943.

Schatz, A., Bugie, E., and Waksman, S. A.: Streptomycin, a substance exhibiting antibiotic activity against Gram-positive and Gram-negative bacteria, Proc. Soc. Exp. Biol. Med., 55:66–69, 1944.

Schmidt, L. H., and Sesler, C. L.: Development of resistance to penicillin by pneumococci, Proc. Soc. Exp. Biol. Med., 52:353–357, 1943.

Schnitzer, R. J., Camagni, L. J., and Buck, M.: Resistance of small colony variants (G-forms) of a *Staphylococcus* toward the bacteriostatic activity of penicillin, Proc. Soc. Exp. Biol. Med., 53:75–78, 1943.

Schoental, R.: The nature of the antibacterial agents present in *Pseudomonas pyocyanea* cultures, Brit. J. Exp. Path., 22:137–147, 1941.

Scudi, J. V., and Jelinek, V. C.: On the inhibition of urease by penicillin, Science, 100:312, 1944.

Shwartzman, G.: On the nature of refractoriness of certain Gram-negative bacteria to penicillin, Science, 101:276, 1945A.

Shwartzman, G.: Concerted antibiotic effect of penicillin, methionine, threonine, and methionine sulfoxide upon brucella, eberthella, salmonella, and shigella, Science, 102:148–150, 1945B.

Smith, L. D.: The bacteriostatic agent of *Penicillium chrysogenum*, J. Frank. Inst., 234:396–402, 1942.

Smith, L. D., and Hay, T.: The effect of penicillin on the growth and morphology of *Staphylococcus aureus*. J. Frank. Inst., 233:598–602, 1942.

Spink, W. W., Ferris, V., and Vivino, J. J.: Antibacterial effect of whole blood upon strains of staphylococci sensitive and resistant to penicillin, Proc. Soc. Exp. Biol. Med., 55:210–213, 1944.

Stuart, L. S., and Harris, T. H.: Bactericidal and fungicidal properties of a crystalline protein isolated from unbleached wheat flour, Cereal Chem., 19:288–300, 1942.

Timonin, M. I.: Another mold with antibacterial activity, Science, 96: 494, 1942.

Tishler, M., Stokes, J. L., Trenner, N. R., and Conn, J. B.: Some properties of gramicidin, J. Biol. Chem., 141:197–206, 1941.

Todd, E. W.: Bacteriolytic action of penicillin, Lancet, 1:74–78, 1945A.

Todd, E. W.: Function of autolytic enzymes in bacteriolysis by penicillin, Lancet, 2:172–175, 1945.

Todd, E. W., Turner, G. S., and Drew, L. G. W.: Temporary character of 'fastness' of staphylocicci to penicillin, Brit. Med. J., 1:111–113, 1945.

T'ung, T., and Frazier, C. N.: The *in vitro* action of penicillin on *H. ducreyi*, Am. J. Syph., Gon., and Ven. Dis. (in press), 1945.

T'ung, T.: Personal communication, 1945.

Turner, J. C., Heath, F. K., and Magasanik, B.: Inhibition of urease by penicillin, Nature, 152:326, 1943.

Ungar, J.: Synergistic effects of para-aminoa benzoic acid and sulfapyridine on penicillin, Nature, 152:245–246, 1943.

Van Bruggen, J. T., Reithel, F. J., Cain, C. K., Katzman, P. A., Doisy, E. A., Muir, R. D., Roberts, E. C., Gaby, W. L., Homan, D. M., and Jones, L. R.: Penicillin B: preparation, purification, and mode of action, J. Biol. Chem., 148:365–378, 1943.

Vilter, S. P., and Spies, T. D.: Vitamin B_6 as an accessory growth factor for *S. albus*, Science, 91:200–201, 1940.

Voegtlin, C., Dyer, H. A., and Leonard, C. S.: Mechanism of action of arsenic upon protoplasm, Pub. Health Rep., 38:1882–1912, 1923.

Waksman, S. A.: Antagonistic interrelationships among microorganisms, Chronica Botanica, 6:145, 1940.

Waksman, S. A.: Nature and mode of action of antibiotic substances, J. Bact., 45:64, 1943.

Waksman, S. A.: Purification and antibacterial activity of fumigacin and clavacin, Science, 99:220–221, 1944.

Waksman, S. A.: Microbial Antagonisms and Antibiotic Substances, New York, 1945. The Commonwealth Fund.

Waksman, S. A., and Bugie, E.: Chaetomin, a new antibiotic produced by *Chaetomium cochliodes*. I. Formation and properties, J. Bact., 48: 527–530, 1944.

Waksman, S. A., Horning, E. S., and Spencer, E. L.: The production of two antibacterial substances, fumigacin and clavacin, Science, 96: 202–203, 1942.

Waksman, S. A., Horning, E. S., and Spencer, E.: Two antagonistic fungi, *Aspergillus fumigatus* and *Aspergillus clavatus*, and their antibiotic substances, J. Bact., 45:233–238, 1943.

Waksman, S. A., and Tishler, M.: The chemical nature of actinomycin, an antimicrobial substance produced by *Actinomyces antibioticus*, J. Biol. Chem., 142:519–528, 1942.

Waksman, S. A., and Woodruff, H. B.: Actinomyces antibioticus, a new soil organism antagonistic to pathogenic and non-pathogenic bacteria, J. Bact., 42:231–249, 1941.

Waksman, S. A., and Woodruff, H. B.: Streptothricin, a new selective bacteriostatic and bactericidal agent, particularly active against Gram-negative bacteria, Proc. Soc. Exp. Biol. Med., 49:207–210, 1942.

Watson, R. F.: Sensitivity of various serological (Lancefield) groups of streptococci to penicillin, Proc. Soc. Exp. Biol. Med., 57:65–69, 1944.

Weindling, R., and Emerson, O. H.: The isolation of a toxic substance from the culture filtrate of *Trichoderma*, Phytopath., 26:1068–70, 1936.

Welsch, M.: La dissolution des germes vivants par les Streptothrix, Compt. rend. Soc. de biol., 124:573–577, 1937.

Welsch, M.: Bactericidal substances from sterile culture media and bacterial cultures, with special reference to the bacteriolytic properties of *Actinomycetes*, J. Bact., 42:801–814, 1941.

Welsch, M.: Bacteriostatic and bacteriolytic properties of *Actinomycetes*, J. Bact. 44:571–588, 1942.

White, E. C., and Hill, J. A.: Studies on antibacterial products formed by molds. I. Aspergillic acid, a product of a strain of *Aspergillus flavus*, J. Bact., 45:433–442, 1943.

Wiesner, B. P.: Bactericidal effects of *Aspergillus clavatus*, Nature, 149: 356–357, 1942.

Wilkins, W. H., and Harris, G. C. M.: Investigations into the production of bacteriostatic substances by fungi. I. Preliminary examination of 100 fungal species, Brit. J. Exp. Path., 23:166–168, 1942.

Wilkins, W. H., and Harris, G. C. M.: Investigation into the production of bacteriostatic substances by fungi. Preliminary examination of a fourth 100 species, all penicillia, Brit. J. Exp. Path., 25:135, 1944.

Williams, R. J., and others: Studies on the vitamin content of tissues I, Univ. of Texas Pub., No. 4137, 1941.

Woodruff, H. B., and Foster, J. W.: Microbiological aspects of penicillin. VII. Bacterial penicillinase, J. Bact., 49:7–11, 1945; Abstr., J. Bact., 47:425–426, 1944. (In Soc. Proc.)

Woolley, D. W., and Longsworth, L. G.: Isolation of an antibiotin factor from egg-white, J. Biol. Chem., 142:285–290, 1942.

Woolley, J. S., and Schmidt, H.: Clinical penicillin assay; an adaptation of the Fleming agar hole method, Am. J. Clin. Path., 15(3): 22, 1945. (In technical section.)

Wrede, F., and Strack, E.: Uber das Pyocyanin, den blauen Farbstoff des Bacillus pyocyaneus, Zeit. fur physiol. chem., 140:1–15, 1924.

Wrede, F., and Strack, E.: Uber das Pyocyanin, den blauen Farbstoff des Bacillus pyocyaneus. IV. Die konstitution und synthese des Pyocyanins, Zeit. fur physiol. chem., 181:58–76, 1929.

CALLING ATTENTION TO:

September, 1945

1. Where Now, Big Boy? The *New Yorker*, as usual avoiding cliches, does well on atomic energy, especially with profile of N.Y. Timesman W. L. Laurence, stimulating science writer (*New Yorker*, August 18, p. 26). Psychology of surrender is well discussed by T. Draper in August *Atlantic*, p. 62. For atomic energy, the fundamental equation is Einstein's E equals Mc^2, where c is the velocity of light (3×10^{10} cm/sec) (*The Meaning of Relativity*, 2d Ed., 1945, 135 pp., Princeton Press, $2). And for this brave new world there is also J. von Neumann and Morgenstern's *Theory of Games and Economic Behavior* (Princeton Press, 1945, 644 pp., $10), and G. Polya's *How to Solve It* (Princeton Press, 1945, 220 pp., $2.50).

2. Symposia and Reviews: S. Warren, B. E. Hall & Co. give neat symposium on radioactive phosphorus in leukemia and polycythemia (*Am. J. Med. Sci.*, 209:701–717, 1945). Note abstract symposium on effects of centrifugal force on animals and man (*J. Phyiol.*, 104: *Proc.* 5–12, June, 1945). Neuropsychiatry symposium (*Brit. Med. Bull.*, 3:1–64, 1945) contains F. M. R. Walshe's account of Queen Square neurology classics and D. Williams clinical application of electroencephalography. Also note symposium of wound healing, burns and shock (*Brit. Med. Bull.*, 3:70–119, 1945). C. B. Friedman reviews pathology of trench foot (*Am. J. Path.*, 21:387, 1945). B. H. Kean and R. G. Grocott review sarcosporidiosis (*Am. J. Path.*, 21:467, 1945). W. J. Tomplinson reviews chronic toxoplasmosis (*J. Clin. Path.*, 15:123, 1945). E. J. Conway analyses physiological significance of inorganicion levels in internal medium of mammals (*Biol. Rev. Cambridge Philo. Soc.*, 20:56, 1945). J. Sinclair calls attention to helpful symposium on genes as physiological agents (*Am. Naturalist*, 29: 289–363, 1945). G. Wald reviews human vision and the spectrum (*Science*, 101:653, June 29, 1945). P. Levine reviews Hr factor and Rh genetic theory (*Ibid.*, 102:1, July 6, 1945). R. F. Pitts reviews renal regulation of acid base balance (*Ibid.*, 102:54, 81, July 20 and 27, 1945). And *Annual Reviews and Physiological Reviews* continue well: J. H. Burn discusses epinephrin and acetylcholin in nervous system; H. F. Blum surveys sunlight effects, and H. N. Harkins reviews burns (*Physiol. Rev.*, 25:377, 483, 531, 1945).

3. Therapeutics: R. A. Peters & Co. find cysteine and methionine useful in recovery from post-arsphenamine jaundice (*Quart. J. Med.*, 14 ns.: 35, 1945). F. Proescher's acridine-sulfa compounds are finally exploited as wound antiseptics by J. McIntosh & Co. (*Lancet*, 2:97, July, 28, 1945). A. M. da Cunha & Co. find penicillin effective in yaws (*Mem. Inst. Oswaldo Cruz*, 41:247, 1945). C. P. G. Wakerley and G. Blum (Berlin M.D.) find UFI, compound of urea and iodine, is helpful when powdered on wounds (*Lancet*, 2:42, July 14, 1945). A. Meyer and D. Teare report

case of fat embolism following electrical convulsion therapy (*B.M.J.*, 2:43, July 14, 1945). F. Himmelweit notes bacteriophage aid in antibiotic action of penicillin against resistant organisms (*Lancet*, 2:104, July 28, 1945). G. C. Linder suggests blood phosphatase as index to dosage of estrogen in prostatic disease (*Clin. Proc., Cape Town P.G. Med. Asso.*, 4:64, 1945). H. F. Shaw indicates value of approach to therapeutics through mode of action of drugs on cells in Blakian style (*Med. J. Austral.*, 1:649, June 30, 1945). V. P. Filatov reports on tissue therapy in cutaneous leishamniasis (*Am. Rev. Sov. Med.*, 2:484, 1945).

4. Odds and: C. Sutherland offers evidence that house-dust allergen is polysacharide (*Med. J. Austral.*, 1:583, June 9, 1945). J. Muniz & G. de Freitas report successful complement fixation diagnostic test for Chagas disease (*Mem. Inst. Oswaldo Cruz*, 41:303, 1944). S. Rose and D. Rabinov report experimental success of electrical anesthesia (*Med. J. Austral.*, 1:657, June 30, 1945). S. Sarkisov (Moscow Brain Inst.) discusses new developments in brain morphophysiology (*Brit. Med. J.*, 2:37, July 14, 1945). W. Feldberg and T. Mann (*J. Physiol.*, 104:8, 1945) confirm D. Nachmansohn and A. L. Machado (*J. Neurophysiol.*, 6:397, 1945) that brain synthesizes acetylcholine anaerobically in presence of adenosinetriphosphate and choline, and add that reduced glutathione and cysteine strongly activate aerobic formation, with inhibition by sugars and Ca, and stimulation by K. E. C. Dodds & Co. (*J. Physiol.*, 104:47–51, 1945) fail to confirm H. G. Wolff & Co. (*J. Clin. Invest.*, 20:63, 1941) that morphine raises pain threshold to heat in man, and report no significant analgesia from diphenylethylamine, although it gives incoördination. B. Benesch shows synthesis of nicotinic acid in human caecum under aerobic conditions and destruction under anaerobic (*Lancet*, 1:718, June 9, 1945).

P. S.—B. de Voto's comments on The University of Texas in August *Harper's* derive from usual sources; like those in *PM* and *Collier's* they are neither inclusive nor conclusive.

Striking October, 1945

1. Bookish: C. W. Heath & Co. worked hard to describe *What People Are: A Study of Normal Young Men* (Harvard Press, Cambridge, '45, 141 pp., $2, and worth it). H. R. Raper's *Man Against Pain; The Epic of Anesthesia* is well, popularly and vigorously written, with merely a few minor inaccuracies and questionable judgments (Prentice-Hall, N.Y., '45, 337 pp., $3.50). A. R. Griffin makes hay *Out of Carnage*, retelling medicine's contributions in war (Soskin, N.Y., '45, 327 pp., $3). R. Caballero analyses Pasteur's philosophical influence (*Pasteur: Estudio Integral de sus Obras el Hombre, el Filosofo, el Creyente*, Univ. Nac. Litoral, Rosario, '45, 147 pp.). H. D. W. Smyth's War Department report on the atom bomb is now available (*Atomic Energy for Military Purposes*, Princeton Press, '45, 264 pp., $2, and full of basic information, so why talk of secrecy?). W. L. Laurence's descriptions of the tests on atom bombing appear serially in *N.Y. Times* beginning Sept. 26, '45. R. N. Ironside & Co.

offer *Aviation Neuro-Psychiatry* (Livingston, Edinburgh, '45, 176 pp., 8s/6d). M. Sherman discusses *Intelligence and Its Deviations* (Ronald Press, N.Y., '45, 286 pp., $3.75). J. M. Hunt writes on *Personality and the Behavior Disorders* (Ronald Press, N.Y., '45, 2 vols. 1242 pp., $10). L. du Nouy offers *Studies in Biophysics: Critical Temperature of Serum (56°)* (Reinhold, N.Y., '45, 183 pp., $3.50). K. A. Pederson reports *Ultracentrifugal Studies on Serum Fractions* (Almquist & Wiksells, Upsala, Sweden, '45, 178 pp., $2.50). R. Lillie wisely theorizes on *General Biology and Philosophy of Organism* (Univ. Chicago Press, '45, $3). I. G. Macy and H. H. Williams cry over *Hidden Hunger: Tragedy of the Unbalanced Diet* (Cattell, Lancaster, '45, 286 pp., $3). O. Temkin gives account of *The Falling Sickness: A History of Epilepsy* (Johns Hopkins Press, Balt., '45, 380 pp., $4). K. Horney notes *Our Inner Conflicts* (Norton, N.Y., '45, 250 pp., $3). H. S. Mustard discusses *Government in Public Health* (Commonwealth, N.Y., 219 pp., '45, $1.50). Have you discovered 19-year-old Tommy Wadelton, Pfc, and his *Silver Buckles on His Knee* (Coward-McCann, N.Y., '45), author of *Army Brat, My Father Is a Quiet Man, and Mother Is a Violent Woman?* Then there's Joe Jackson's *Viking Portable Murder Book* (Viking Press, N.Y., '45, 570 pp., $2), if you're interested.

2. Fascistish? Interesting proposals for Federal support (and control?) of scientific research and education by V. Bush (Science: *The Endless Frontier*, U.S. Govt. Printing Office, Washington, '45, 184 pp.), L. Chalkley (*Science*, 102:289, Sept. 21, '45), W. Kaempfert (*N.Y. Times*, July 22 and later), and Senators Magnuson, Kilgore & Co. Is there danger of scientific orthodoxy by continued control of small eastern group of national scientific societies, scientific journals, and now of national funds for support of scientific research and education. For English reaction note editorial on freedom of scientific intercourse (*Nature*, 156:215, Aug. 25, '45).

3. Schoolish: Stimulating symposium on medical education (*Lancet*, 2:225–237, Aug. 25, '45). J. J. Izquierdo confirms our finding of defects in premedic physics and chemistry training, and gives fine appreciation of W. B. Cannon (*Gac. Med. Mex.*, 75:101, 132, '45). Some medical students say what they think about medical schools (*J. Asso. Am. Med. Coll.*, 20:296, '45).

4. Antibiotish: G. Lapage reviews recent reports on uses and limitations of penicillin (*Nature*, 156, 244, Aug. 25, '45). A. L. Barach & Co. find tablespoonful aluminum hydroxide gives practical oral adminstration of penicillin (*Science*, 102:247, Sept. 7, '45). J. C. Lewis & Co. find formaldehyde treated gramicidin loses hemolytic and toxic action but not antibiotic (*Ibid.*, p. 274, Sept. 14, '45). D. Perlstein and A. J. Liebmann announce production of anti-penicillinase immune sera (*Science*, 102:197, Aug. 24, '45). W. F. Elias & Co. bacteriostatic activity in human sera (*Ibid.*, p. 223, Aug. 31, '45).

5. Worthish: Note symposium on electroshock therapy (*J. Nerv. Ment. Dis.*, 102:221–247, '45). H. E. Malosetti describes suboccipital encepha-

lography (*Anal. Fac. Med. Montevideo*, 30:341–526, '45). L. A. Surraco and J. Lockhart find IV injection of magnesium hyposulfite useful in causalgic symptoms of hot painful foot syndrome (*Ibid.*, p. 293). E. B. Astwood & Co. note reversible motor paralysis after repeated dithiobiuret (*Science*, 102:196, Aug. 24, '45). D. Nachmansohn and H. M. John find choline acetylating enzyme system in nerve axon and suggest relation to phosphate bond energy and nerve action potential (*Ibid.*, p. 250, Sept. 7, '45). O. W. Smith and G. V. Smith report a fibrinolytic enzyme in menstruation and pregnancy toxemia (*Ibid.*, p. 253). H. Keil notes biochemorphology of Anacardiaceae (*Ibid.*, p. 279, Sept. 14, '45).

SCHIZOID NOVEMBER, 1945

1. SYMPOSIA: Excellent one on cardiac output by A. F. Cournand, W. F. Hamilton, I. Starr, J. L. Nickerson, J. McMichael, and D. W. Richards (*Fed. Proc.*, 4:183–220, '45). Then go on to W. F. Hamilton & Co.'s neat studies on propagation velocity of arterial pulse wave, cardiac ejection curves, and ballistocardiographic forces (*Am. J. Physiol.*, 144:521–570, '45). Note papers on intervertebral disc and lumbago by E. J. Crisp, B. H. Burns, R. H. Young, and J. Cyriax (*Lancet*, 249:422–429, Oct. 6, '45). Important series on physics in medicine by W. V. Mayneord, H. Hurst, G. E. Donovan, D. S. Evans, K. Mendelssohn, and A. H. S. Holbourn (*Brit. Med. Bull.*, 3:129–151, '45), including interesting historical notes. Note potential competition from R. Geigy & Co. on recent researches on malaria in Switzerland (*Acta Tropica*, 2:1–159, '45).

2. ETHICS: P. Romanell takes "Ethicogenesis" for a technical hay-ride (*Sci. Month.*, 61:293, '45; for other cracks see p. 329). O. L. Reiser looks to the *Promise of Scientific Humanism* (Creative Age Press, N.Y., 382 pp., $4), and proposes an institute of scientific humanism (*Philos. Sci.*, 12:45, '45). Meanwhile G. Sarton publishes *Isis*, and too few help him. W. A. R. Leys writes on *Ethics and Social Policy* (Prentice-Hall, N.Y., 522 pp., $3.50). A. Edel discusses key concepts in ethical theory (*Philos. Rev.*, 12:260, '45). A. Robertson offers *Morals in World History* (C. A. Watts, London, '45, 126 pp., $2.50). C. L. Stevenson joins *Ethics and Language* (Yale, New Haven, '45, 350 pp., $6). A. M. Schwitalla, S.J., undertakes to edit *The Linacre Quarterly*, a journal of the philosophy and ethics of medical practice from the orthodox standpoint. D. M. Emmet describes *Nature of Metaphysical Thinking* (Macmillan, '45, 238 pp., $2.50) which contains statements, if you're interested, about the real which transcends experience! Vale to B. Malinowski who closes career with *Dynamics of Culture Change* (Yale, New Haven, '45), and *A Scientific Theory of Culture* (Univ. No. Carolina, Chapel Hill, '45, 228 pp., $4). A. G. Ramsperger notes misplaced modesty of scientists (*Antioch Review*, Winter, '45, p. 581). A. Dresden's *Mathematics as an Intercultural Bridge* remains unpublished from 6th conference on Science, Philosophy and Religion, and it's pertinent. Lots of physicists seem willing to accept responsibilities of

implications of current scientific work; why not a series of seminars, conferences, and congresses with biologists, physicians, sociologists, philosophers, and policy leaders? Note A. R. Zhebrak's comment (*Science*, 102: 357, Oct. 5, '45). British scientists propose international science coöperative service (*Nature*, 156:401, Oct. 6, '45).

3. ANTIBIOTICS: M. I. Smith and W. T. McClosky find streptomycin has chemotherapeutic index ten times that of promin in experimental TB (*Pub. Health Rep.*, 60:1129, Sept. 28, '45). H. A. Zintel & Co. report streptomycin block levels well maintained with slow urinary excretion, low toxicity, now side actions, wide tissue distribution, poor oral absorption (*Am. J. Med. Sci.*, 210: 421, '45). B. A. Johnson & Co. obtain "bacitracin" from B. subtilis, a water soluble, non-toxic, heat stable, effective agent vs. hemolytic streptococci and staphlococci (*Science*, 102:376, Oct. 12, '45).

4. NEWS: G. R. Herrmann and P. A. Rockwell report on reversable and irreversable liver disease in reference to choline (*Texas St. J. Med.*, 41:288, '45). D. E. Clark & Co. conclude that lipotropic effect of lipocaic is due to some factor other than choline, methionine or protein action (*Am. J. Physiol.*, 144:620, '45). B. A. Houssay and J. Sara observe that thyroid enhances toxic and diabetogenic action of alloxan (*Rev. Soc. Argent. Biol.*, 21:81, '45). E. de Robertis and W. W. Nowinski find proteolytic effect of pathological thyroid greater than normal (*Ibid.*, p. 120). A. Van Harreveld notes re-innervation of denervated fibers by adjacent functioning motor groups (*Am. J. Physiol.*, 144:477, '45). A. E. Ritchie reviews physiology of peripheral nerve injury (*Edin. Med. Surg. J.*, 52:289, '45). N. W. Shock and W. H. Sebrell observe increased work output (from frog muscle) after thiamine pyrophosphate (*Proc. Soc. Exp. Biol. Med.*, 59:212, '45). R. Gubner and J. DiPalma say that glycine increases peripheral blood flow (*Ibid.*, 170). E. Gellhorn reports recovery of inhibited conditioned reflexes after shock therapy (*Ibid.*, 155). H. L. Hamilton surveys biochemorphology of p-aminobenzoic acid inhibition of rickettsial growth (*Ibid.*, 220). J. B. de C. M. Saunders and W. Haymaker compare sulfa drug toxicity on chick brain cultures (*Ibid.*, 306). Enjoyable is C. D. O'Malley and J. B. de C. M. Saunders' note on St. Apollonia (*J. Am. Coll. Dent.*, 10:101, '45). H. L. Segal propose anion exchange resin (polyamine formaldehyde) to control gastro-enteric pH (*GastroEnt.*, 4:484, '45). W. H. Lewis and A. G. Richards find D.D.T. non-toxic to cells in culture (*Science*, 102, 330, Sept. 28, '45).

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